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Extraction and Separation of Phytoestrogens from Over-the-Counter Supplements

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Extraction and Separation of Phytoestrogens from Over-the-Counter Supplements

Graham Grinnell

Worcester Polytechnic Institute

June 20, 2013

Abstract

This study involves the analysis of over-the-counter phytoestrogen supplements due to their anti-proliferative effects on breast cancer cells. Phytoestrogens have been used as alternatives to traditional hormone therapy in postmenopausal women. TLC and HPLC were employed to optimize extraction and separation of these compounds from over-the-counter supplements. Testing of Promensil, Black Cohosh, and Soy Isoflavone supplements revealed that significant anti-proliferative effects observed in Promensil and Black Cohosh may be attributable to their combination of the phytoestrogens daidzein and biochanin A.

Acknowledgements

Sincerest gratitude goes out to all of the following:

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If anyone is missing in the list above, please consider yourselves thoroughly thanked as well. This project would not have succeeded if it weren't for everyone involved generously donating their time and resources.

Thank you!

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Background

This study is part of an ongoing work in the Biology and Biotechnology Project Lab at Worcester Polytechnic Institute. Beginning in 2007 with Caron's thesis, in which it was found that phytoestrogens present in the over-the-counter supplement Promensil have a profound inhibitory effect on the proliferation of MCF7 breast cancer cell lines (Caron, 2007), several successive studies have focused on these steroidal compounds and their effects on breast and prostate cancer cells. To determine what compounds in isoflavone supplements invoke these reactions, it is imperative that they be extracted from the supplements and isolated for further testing. This may be achieved through the use of separatory techniques such as chromatography. The first chapter of this study explores methods of extraction and analyzes them through thin-layer chromatography; the second chapter employs high-performance liquid chromatography to identify major compounds of interest from the separated constituents of extraction mixtures.

Estrogens are a class of steroidal hormones produced in mammals which promote differentiation of female reproductive organ tissues. They are mainly produced in the ovaries and adrenal glands; however, trace amounts are also produced elsewhere in the body. Three of these estrogens occur naturally within humans: 17β-estradiol, estrone, and estriol (Turner, 1971).

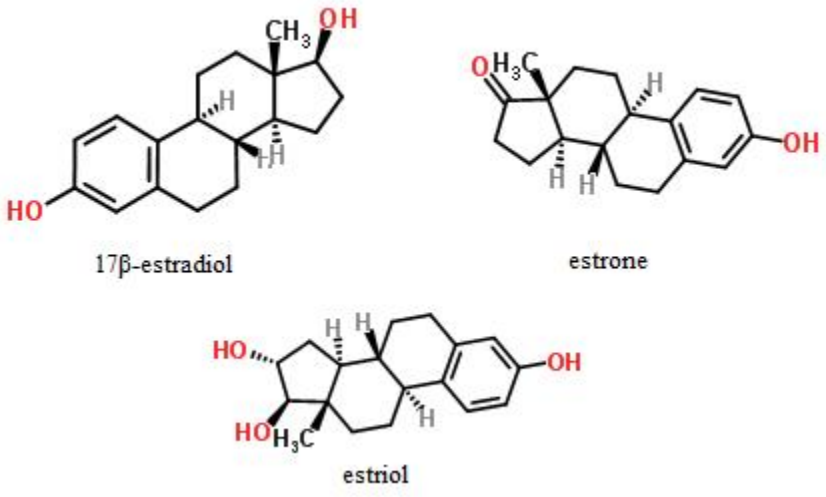


Figure 1- *The three naturally-occurring human estrogens and their structures (chemspider.com, 2013).*

17β-estradiol is generally the most active of the three, and may be converted to the less potent estrone or estriol by estrogen-metabolizing enzymes to regulate hormone levels. Other metabolite products of these estrogens may also be present within the body at times (Vaskivuo, 2005). When a female reaches menopause her production of estrogens in the ovaries falls drastically, resulting in symptoms such as menopausal flushes, increased risk of cardiovascular diseases, and a decrease in bone density which may lead to osteoporosis (Hadley, 1988; Hulley,

1998). To relieve these symptoms, hormone replacement therapy may be employed. Unfortunately there are health risks associated with this form of therapy, such as an increased risk of pulmonary embolism and possibly even breast cancer; this being the case, many women have turned to over-the-counter phytoestrogen supplements rather than traditional hormone replacement therapy (Hulley, 1998; David, 2001).

Phytoestrogens (Fig. 2), another name for the isoflavones, are a class of plant-produced steroidal compounds which, in their native organisms, serve the purpose of hormones to induce flowering, growth, and healing of wounds (Turner, 1971). They are most prevalent in the leguminous plants including but not limited to beans, peas, clovers, and soy (Harjo, 2007).

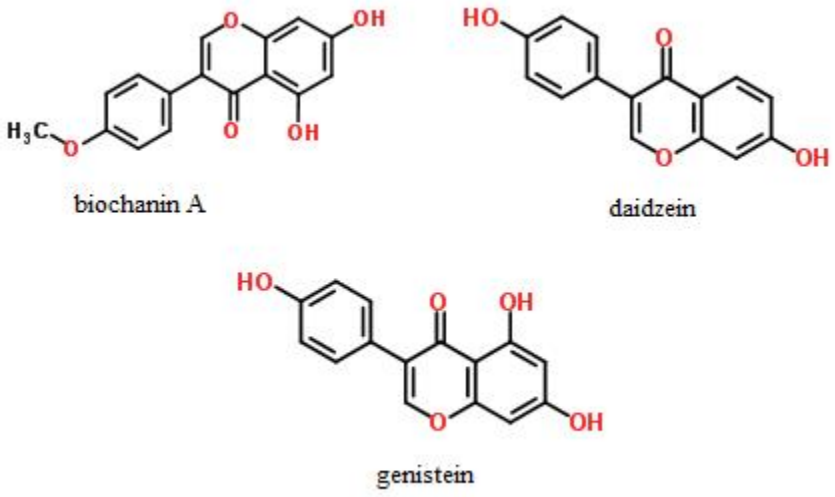


Figure 2- *Examples of phytoestrogens and their structures (chemspider.com, 2013).*

In the human body, however, phytoestrogens have many intriguing effects and health benefits. Their structural similarities to the human estrogens allow them to bind to estrogen receptors and in doing so provide much of the same effect as estrogens (Vacek, 2008). Due to the structural similarities, they exhibit competitive binding with natural estrogens and therefore they are most effective in low-estrogen environments such as those found in postmenopausal women (Messina, 1999). A synthetic isoflavone, ipriflavone, displayed potential for the prevention of osteoporosis by increasing bone mineral density in postmenopausal women (Messina, 1999). In 1993 Coward *et al* observed an overall lower incidence of breast cancer in premenopausal Asian women, which may be linked to diets containing high amounts of soy product rich in phytoestrogens (Coward, 1993). This may be attributable to the antagonistic qualities of phytoestrogens: estrogen-receptor positive breast cancer would be inhibited by their competitive binding with estrogens (Zajchowski, 1993). These beneficial effects have led to a large market for over-the-counter phytoestrogen supplements, but the possibility of these compounds acting as estrogen agonists when in the body leads one to believe they may have the same downsides and risks as

traditional hormone replacement therapy, especially since they are unregulated from lot to lot (Setchell, 2001).

CHAPTER I: Supplement Extraction and Thin-Layer Chromatography

Introduction

The extraction and isolation of phytoestrogens has been studied for well over a century: a study by Perkin and Newbury in 1899 was the first to isolate genistein from the legume *Genista tinctoria*, from which the name of the compound was derived (Perkin, 1899). Their experiment involved an extraction first in acid, then an alcohol which is assumed to be methanol: they do not clarify in their writing. While it was primarily a study of the coloring compounds present in the legume, this inadvertent discovery of a new compound would facilitate interest in the further extraction and categorization of phytoestrogens. In 1941 Walter experimented on soybeans to determine further physical and chemical properties of genistein while also refining the isolation procedure (Walter, 1941).The solvent of choice remained methanol, but experimentation with different solvents would become an important part of the phytoestrogen extraction process.

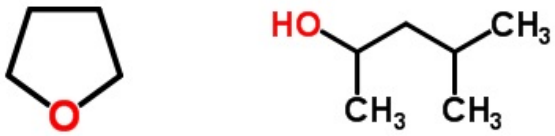
Farmakalidis and Murphy found that both genistein and daidzein could be extracted from soyflakes through the use of acetone and isolated from the extract through silica gel column chromatography using an elution mixture of 9:1 chloroform and methanol (Farmakalidis, 1985). Murphy later tested acetonitrile, acetone, ethanol, and methanol extractions of soy and found acetonitrile to be the most efficient for extracting phytoestrogens (Murphy, 2002). Other experiments have showed that hexane, 2-propanol, isopropanol, and other relatively plentiful organic solvents were viable alternatives to methanol or ethanol extractions (Vacek, 2008).

Setchell *et al* used methanol in an attempt to extract as much phytoestrogen content as possible from 33 varying over-the-counter supplements. Since these supplements are undefined, unregulated mixtures of plants, the group's intent was to verify how accurate the producers' claims were regarding their products' phytoestrogen content and therefore an all-inclusive method of extraction was necessary (Setchell, 2001). Caron later used the same method in extracting genistein from the over-the-counter product Promensil (Natrol); the resulting methanol extract was observed to have anti-proliferative effects on MCF7 breast cancer cell lines (Caron, 2007).

As a result, Promensil, as well as two other over-the-counter supplements, Soy Isoflavones (Natrol) derived from soy plants and Black Cohosh (Solaray) derived from the black cohosh plant *Cimicifuga racemosa*, will be the focus of this study. The Promensil packaging states that one dose contains 40 mg of isoflavones without indicating any compounds in particular. Natrol claims its Soy Isoflavones contains 20 mg of genistein, 17.5 mg of daidzein, 6.25 mg glycitein, and 6.25 mg "other isoflavones" per dose. Solaray's Black Cohosh packaging does not list any specific phytoestrogen content, simply stating "Black Cohosh is known for its phytoestrogen properties." The high variability in the nature of these plant products means

phytoestrogen content may differ significantly between batches if the source plants are not kept in highly controlled environments. Even then, variables such as the seasons may factor in to phytoestrogen content from batch to batch.

Particular phytoestrogens or supplements may require more efficient extraction media than others. To provide a more optimal and efficient extraction method for the three supplements in question, various organic solvents were tested for their extraction efficiency relative to methanol; this would also determine viable alternatives for different situations. Besides methanol, tetrahydrofuran and 4-methyl-2-pentanol (Fig. 3) were selected for extraction testing. THF was selected for its significant polar properties which would better facilitate extraction of compounds such as phytoestrogens. Conversely, 4-methyl-2-pentanol was selected in the interest of seeing whether side chains on a solvent compound might inhibit extraction efficiency.



tetrahydrofuran 4-methyl-2-pentanol

Figure 3- *Selected alternative extraction solvents and their structures (chemspider.com, 2013).*

Materials and Methods

Extraction of phytoestrogens from supplements:

Supplement extraction was performed according to Caron's method (Caron, 2007). Extraction media were prepared as follows: 80% methanol (V/V in H_2O), 80% tetrahydrofuran (V/V in H_2O), and pure 4-methyl-2-pentanol. Three different over-the-counter supplements were chosen for extractions: Promensil (Natrol), Black Cohosh (Solaray) supplements, and Soy Isoflavones (Natrol) supplements. For each extraction, four tablets of the corresponding supplement were ground by mortar and pestle before being added to 80 mL extraction medium. The solutions were then refluxed with a water-jacketed condenser for 1 hour before being filtered and stored at 4°C. An 80% 4-methyl-2-pentanol (V/V in H_2O) solution was also prepared to determine whether isoflavones would extract more readily to the organic or aqueous phases. This was only tested with Promensil.

Determination of proper spot volumes and plate composition for TLC:

This initial test used two alumina TLC plates running concurrently. Methanol extractions of each of the three supplements were tested; a micropipettor was used to spot one plate with 20 µL and the second plate with 10 µL of each extraction. TLC was then run for 90 minutes using an 8:3:1 chloroform/methanol/acetonitrile mobile phase as detailed in Caron's experiment. A second test was run to analyze the viability of silica with gypsum versus alumina plates, as well as test smaller load sizes. One alumina plate and one silica with gypsum plate were loaded with the methanol extractions and run using the solvent described above; one sample was first centrifuged at 3000 RPM for 5 minutes and the supernatant was used for its spot. Load sizes were 10 µL, 5 µL, 3 µL, and 1 µL of the extract before centrifugation and 5 µL of the centrifuged sample. All plates were allowed to dry, then analyzed under short-wave UV light. Visible spots were marked for identification by retention factor (R_f) values.

Determination of TLC clarity among all extracts:

TLC tests were then run using 5 µL loads of all extraction mixtures on silica with gypsum plates. Again, the samples were centrifuged and the supernatant used for spotting. The 8:3:1 chloroform/methanol/acetonitrile mobile phase was used again and the assay was run for 90 minutes to allow the solvent front to move near the top of the plate. The plates were then allowed to dry. Plates were again visualized and marked under short-wave UV. Two further test plates were run to confirm the system's consistency.

Identification of phytoestrogens in supplements by TLC:

Pure biochanin A (Alfa Aesar), daidzein (Enzo Life Sciences), and genistein (Enzo Life Sciences) were obtained for use as pure phytoestrogen standards. These were used to prepare 10 mL solutions of each in 20 µM concentrations in methanol. The three standard solutions were then run concurrently with all extraction mixtures. All extracts were again centrifuged before loading the supernatants; the standards were not. Again, 5 µL spots were used and TLC was run for 90 minutes with the 8:3:1 chloroform/methanol/acetonitrile mobile phase. The plates were analyzed and marked under short-wave UV.

Testing the TLC assay using silica F_{254} plates:

Caron's TLC experiment used silica F_{254} plates; for consistency and to improve clarity of the TLC assay, these were chosen to be used for further testing rather than silica with gypsum plates. One test plate was run with all standards and extracts. A spot test was employed to ensure the standards could be observed on the silica F_{254} plates using short-wave UV. Both centrifuged and non-centrifuged samples were spotted.

Determination of a solvent system to improve TLC clarity:

After the spot test, it was observed that the standards were in fact present on the TLC plates, but they were along the solvent front. In an attempt to remedy this situation several small-scale solvent system tests were run to determine a more optimal ratio of solvents. The amount of chloroform was increased due to it having the lowest elutropic value (Hofmann, 1977) of the three solvents, then acetonitrile was also increased to effectively decrease the overall amount of methanol in the system. 12:3:1 and 12:3:2 mixtures were tested. Another small-scale test was used with 12:3:1, 12:2:1, and 12:1:1 ratios on silica with gypsum to test the effect of varying amounts of methanol, which had the highest elutropic value of the three solvents (Hofmann, 1977).

Identification of phytoestrogens present in extracts:

Two TLC plates were run using the 12:2:1 chloroform/methanol/acetonitrile mobile phase identified in the previous test. The plates were again run for 90 minutes, then allowed to dry before being visualized under short-wave UV. R_f values for all bands were calculated to confirm consistency.

Results and Discussion

Initial tests were run using Caron's 8:3:1 chloroform/methanol/acetonitrile solvent mixture. Alumina plates were the first available and were used to determine a proper load size (spot volume) for the TLC assay. Two plates were run concurrently: one with 10 µL spots and the second with 20 µL. The resultant plates (Fig. 4) displayed large smudges, suggesting the load sizes were much too large.

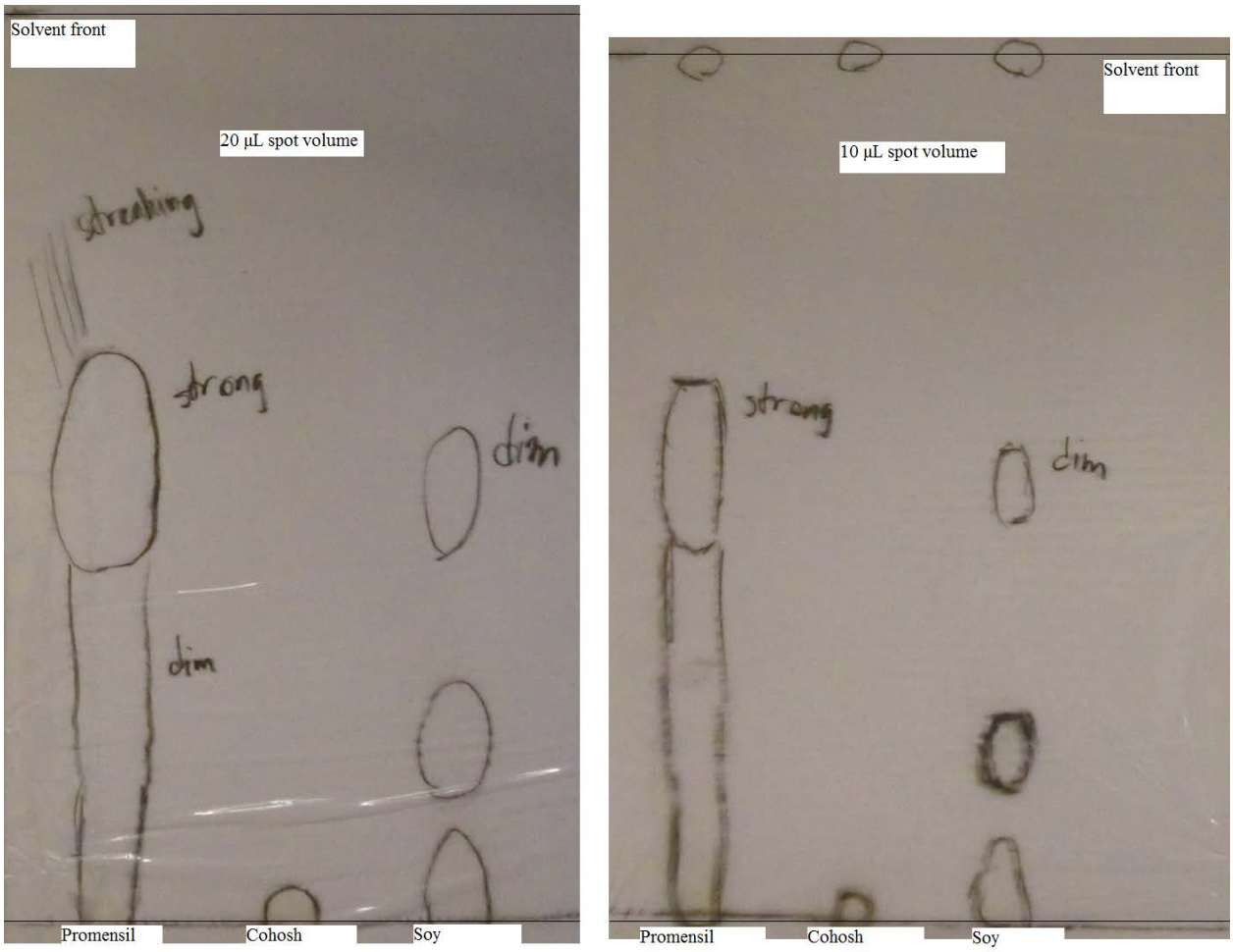


***Figure 4**- Initial spot volume tests using alumina plates. Load volumes of 20 µL (left plate) and 10 µL (right plate) were tested for visibility.*

To find a proper load size and test the viability of alumina plates versus silica with gypsum plates, the next test was run with one alumina plate and one silica with gypsum plate concurrently. Results displayed higher clarity and better resolution on the silica with gypsum plate (Fig. 6), on which there were evident bands. The alumina plate (Fig. 5) again had smudging in every lane. It was determined from there that the silica with gypsum plates would be used in later assays and that samples would be spun down before spotting them in 5 µL volumes.

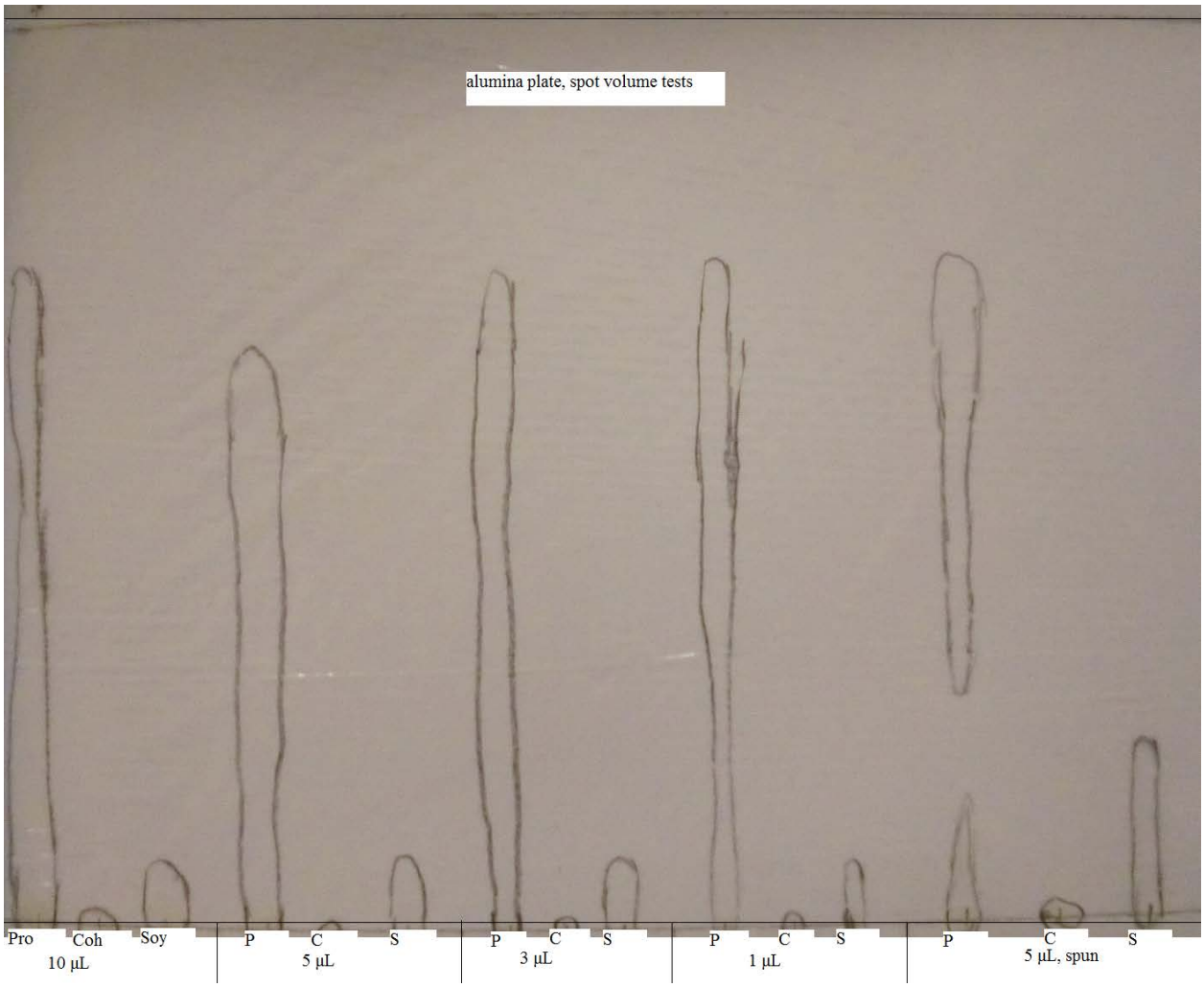


Figure 5*-Further spot volume tests performed on an alumina plate. Load volumes were 10, 5, 3, and 1 µL; the rightmost lane was loaded with 5µL supernatant from a centrifuged sample.*

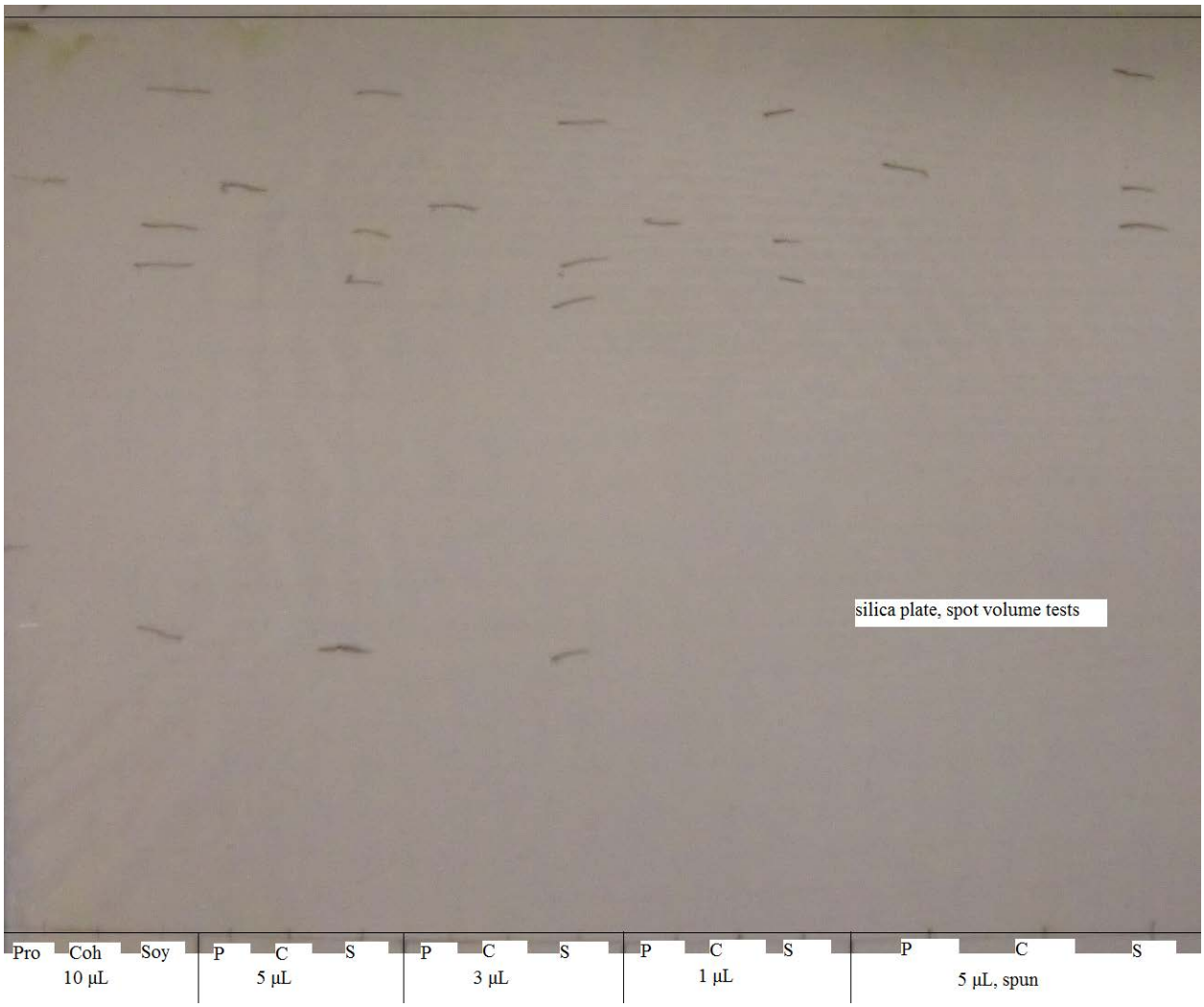


Figure 6*- The same spot volume tests as Fig. 5 on a silica with gypsum TLC plate.*

Two test plates were then run (Figs. 7 and 8) to see how all the extracts would resolve using the determined methods. Bands were observed, yet those in the methanol extraction lanes were inconsistent with those seen in the preliminary tests. To eliminate variables the TLC chamber was cleaned and a fresh solvent mixture was made. A third test was run and excessive smudging was observed (Fig. 9): it was later determined the chloroform used in the second mixture of solvent was contaminated.

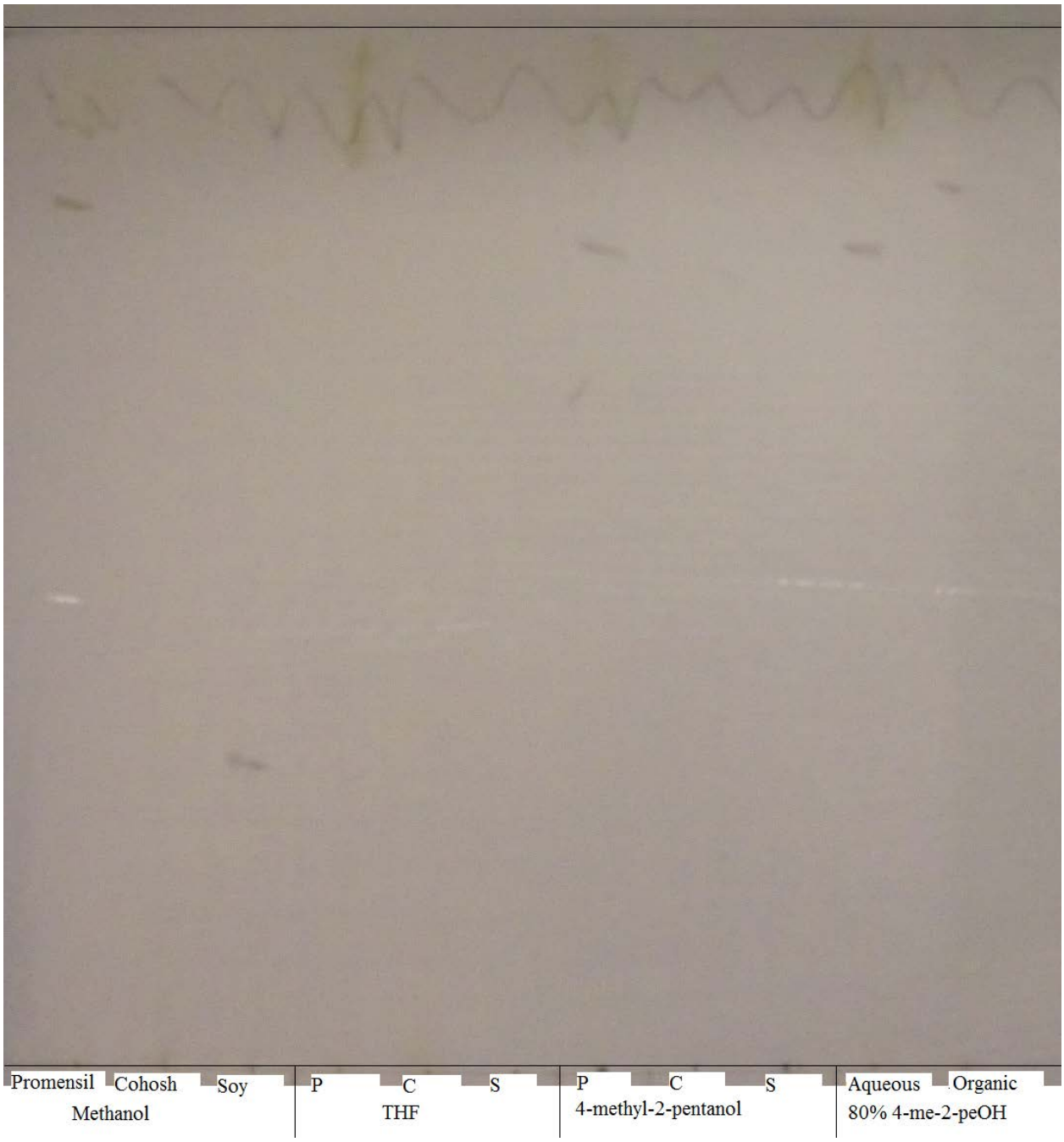


Figure 7- *Extract test plate #1. THF = tetrahydrofuran. Aqueous and Organic refer to the respective phases in the 80% 4-methyl-2-pentanol extraction.*

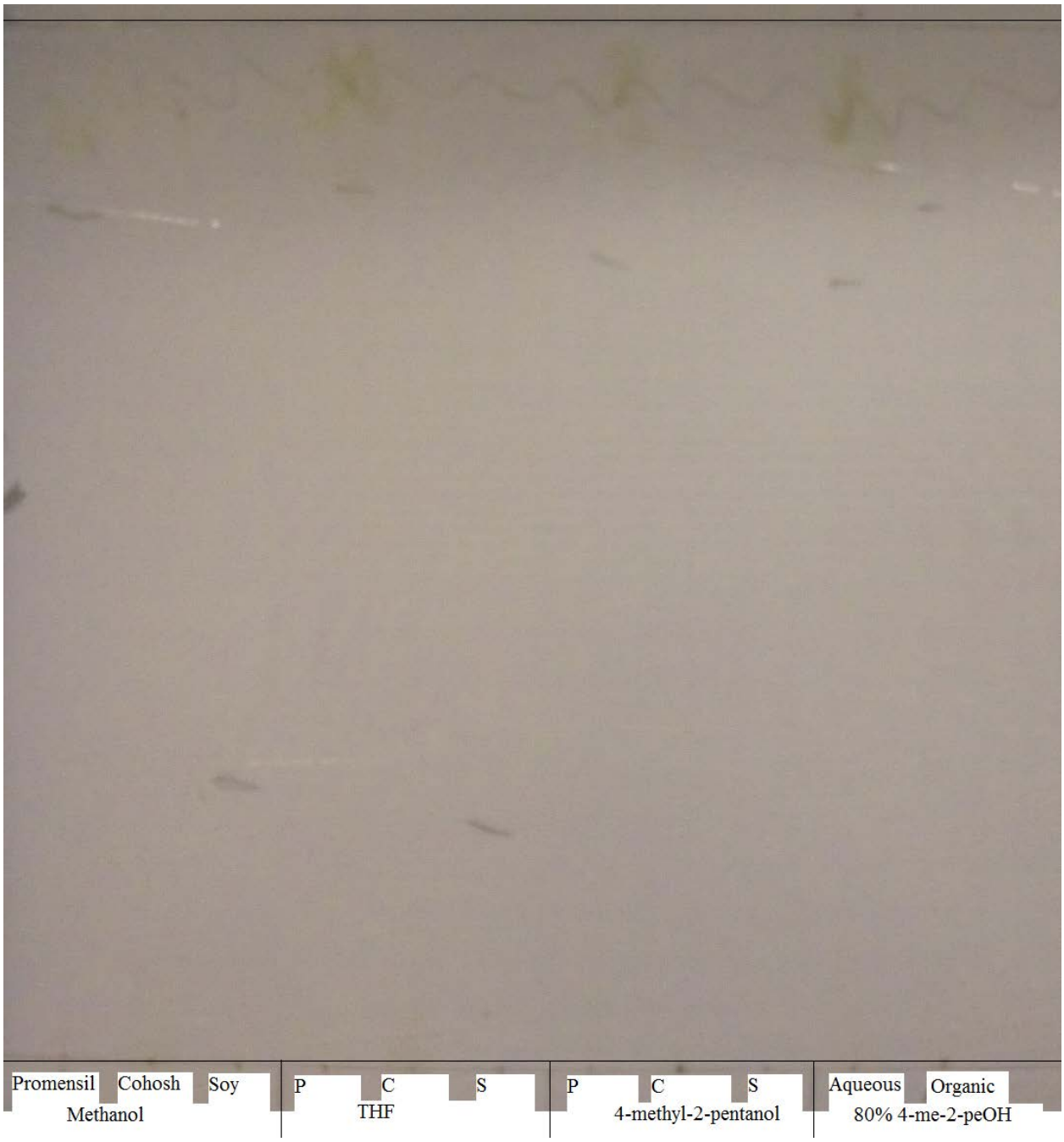


Figure 8*- Extract test plate #2, run identically to Fig. 7.*

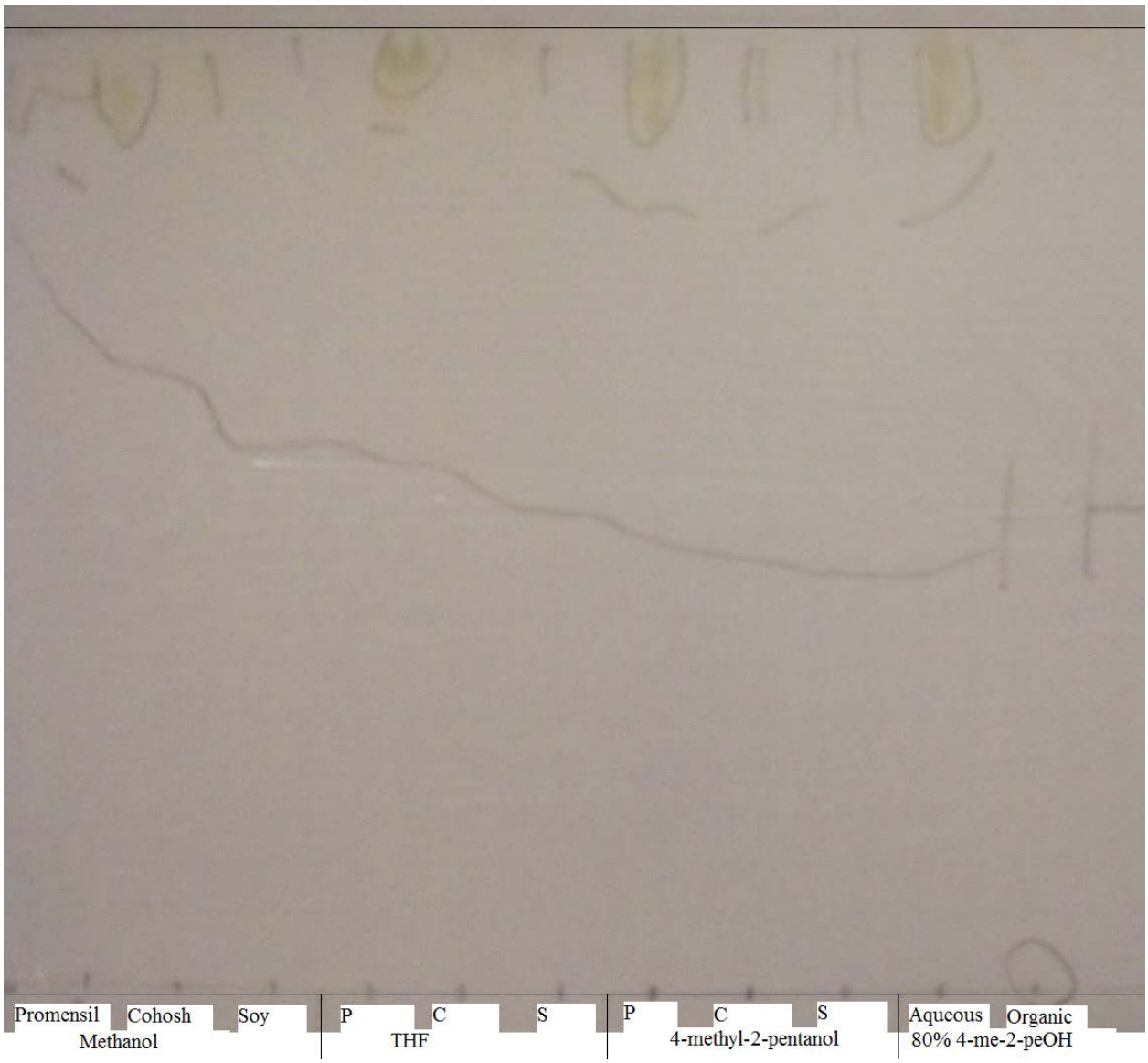


Figure 9*- Extract test plate #3, run identically to Figs. 7 and 8 but with fresh solvent. Excessive smudging was attributed to contaminated chloroform used in the new solvent.*

After obtaining more chloroform for the solvent the test plates were run again; this time, they exhibited relatively clear, albeit few, bands (Figs. 10 and 11). Consistency was also observed visually between the two plates, including the smear at the beginning of the 4-methyl-2-pentanol aqueous phase lane. It was then assumed the TLC assay methodology was working correctly and phytoestrogen standards could be introduced.

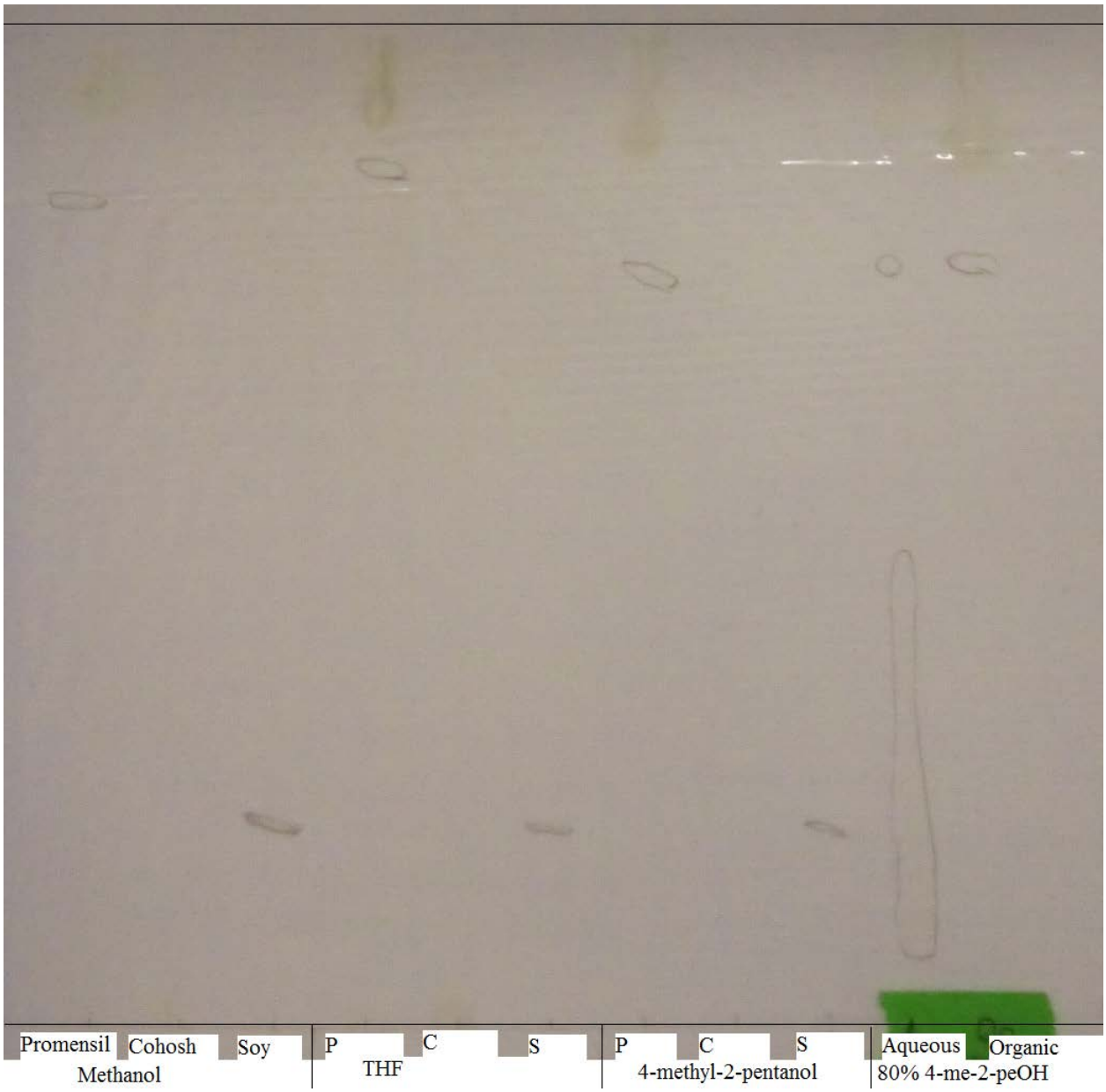


Figure 10*- Test plate #1 to determine consistency of the TLC methodology.*

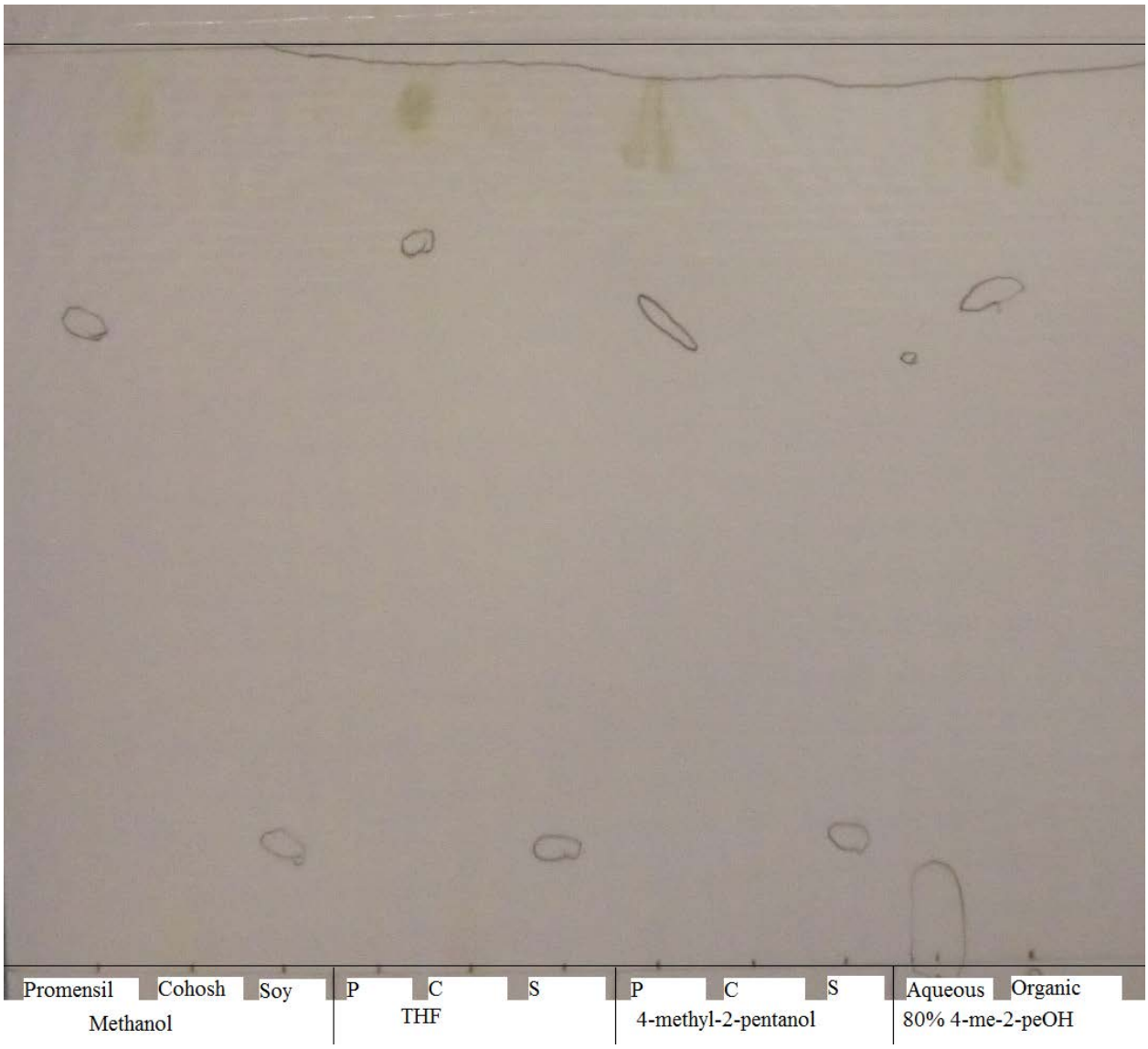


Figure 11- *Test plate #2 to determine consistency of the TLC methodology. Visualized spots were comparable to those seen in Fig. 10, suggesting consistency in the method.*

Three pure phytoestrogen standards were prepared in 20 µM concentrations in methanol: biochanin A, daidzein, and genistein. These were run concurrently with the extraction samples to determine the relative presence of phytoestrogens in the extracts (Fig. 12). Upon viewing the finished plate under UV, however, the standards could not be visualized.

Figure 12- *Phytoestrogen standards test plate #1 on silica with gypsum. No spots were observed in the standard lane.*

A second test plate was run using UV-active alumina to see if there was any significant difference; this only resulted in the smudging and streaking exhibited in earlier tests using alumina, although there was a smudge showing the presence of daidzein (Fig. 13).

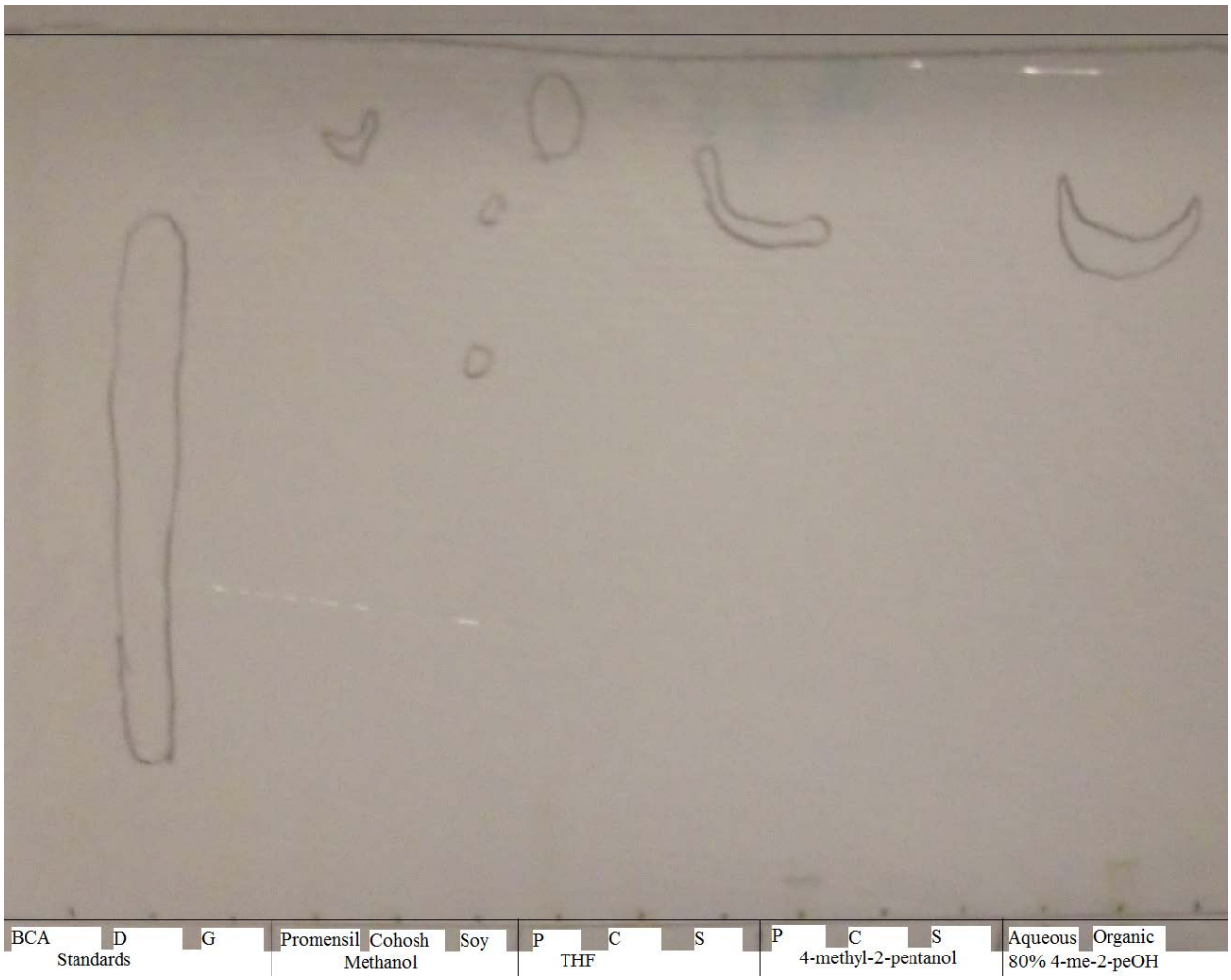


Figure 13- *Phytoestrogen standards test plate #2 on alumina. Despite excessive smudging of spots, daidzein presence was observed in the phytoestrogen standard lane.*

To eliminate further variables, the TLC plates were changed for the same silica F_{254} UV-active plates used in Caron's assays (Caron, 2007). One run was carried out to test the new plates (Fig. 14). A small section of one plate was then used for a spot test without being chromatographed to ensure the phytoestrogen standards could be visualized on the plates. All three spots, whether centrifuged or not, could be visualized under short-wave UV (Fig. 15). This confirmed the standards were in fact visible on the silica F_{254} plates.

Figure 14*- Phytoestrogen standards test plate #3 on silica F_{254}. Spot clarity was significantly improved from that of previous assays.*

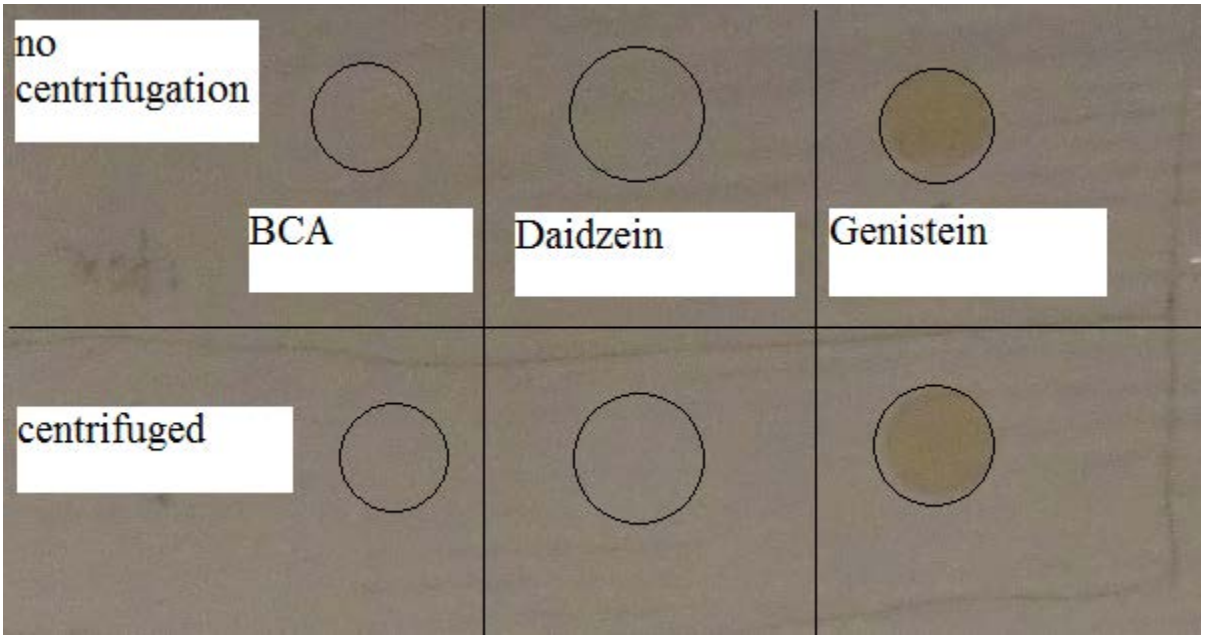


Figure 15*- Phytoestrogen standard spot tests. Under short-wave UV, clear spots appeared for all phytoestrogen standards.*

A second test plate was run to confirm the phytoestrogen standards remained visible on the silica F_{254} plates after being chromatographed (Fig. 16). These tests determined the standards had been traveling with the solvent front; therefore the solvent system would require modification.

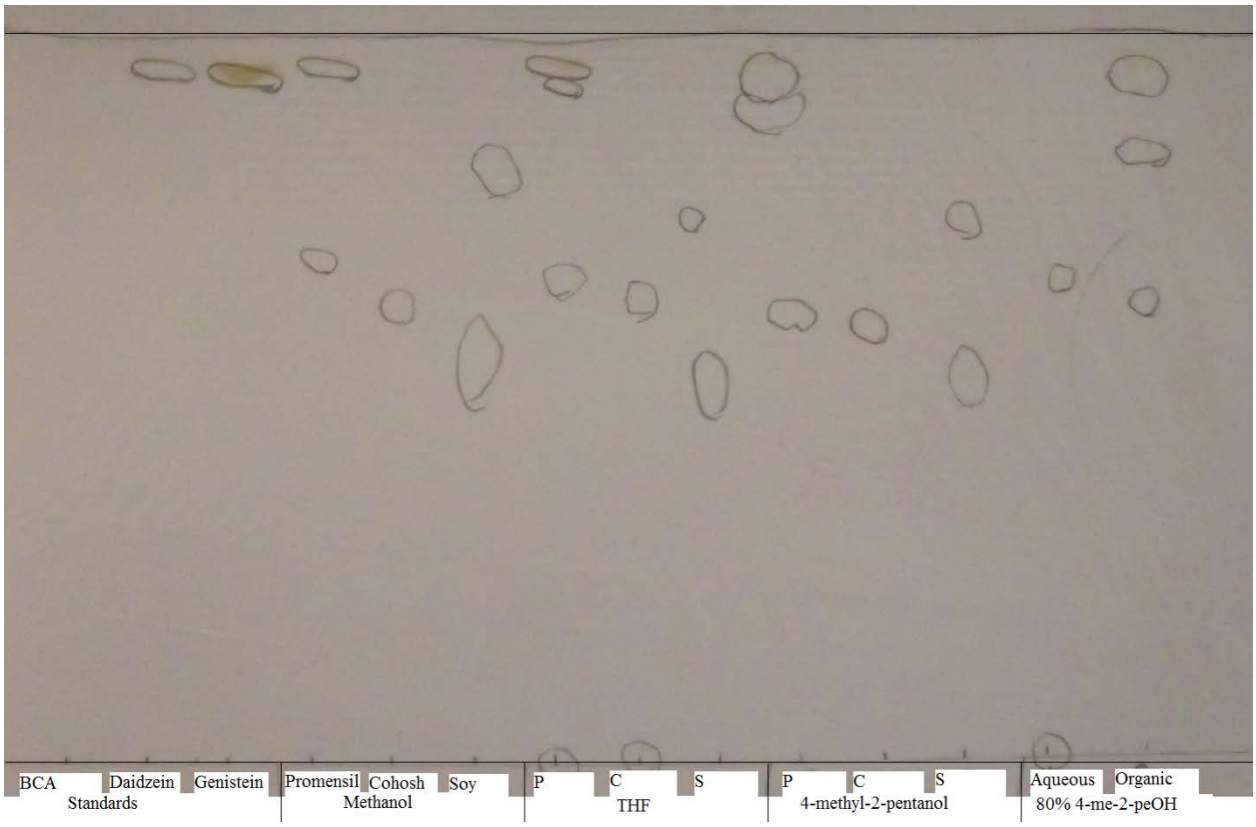


Figure 16*- Silica F_{254}, second test plate. This confirmed the standards were running along with the solvent front and the solvent ratio would require modification.*

Following these tests, two full-scale tests were run to modify the relative amount of methanol in the mixture. The first plate was run with an increased ratio of chloroform to create a 12:3:1 chloroform/methanol/acetonitrile mix. Results displayed only a small effect on the movement of the phytoestrogen standards (Fig. 17).

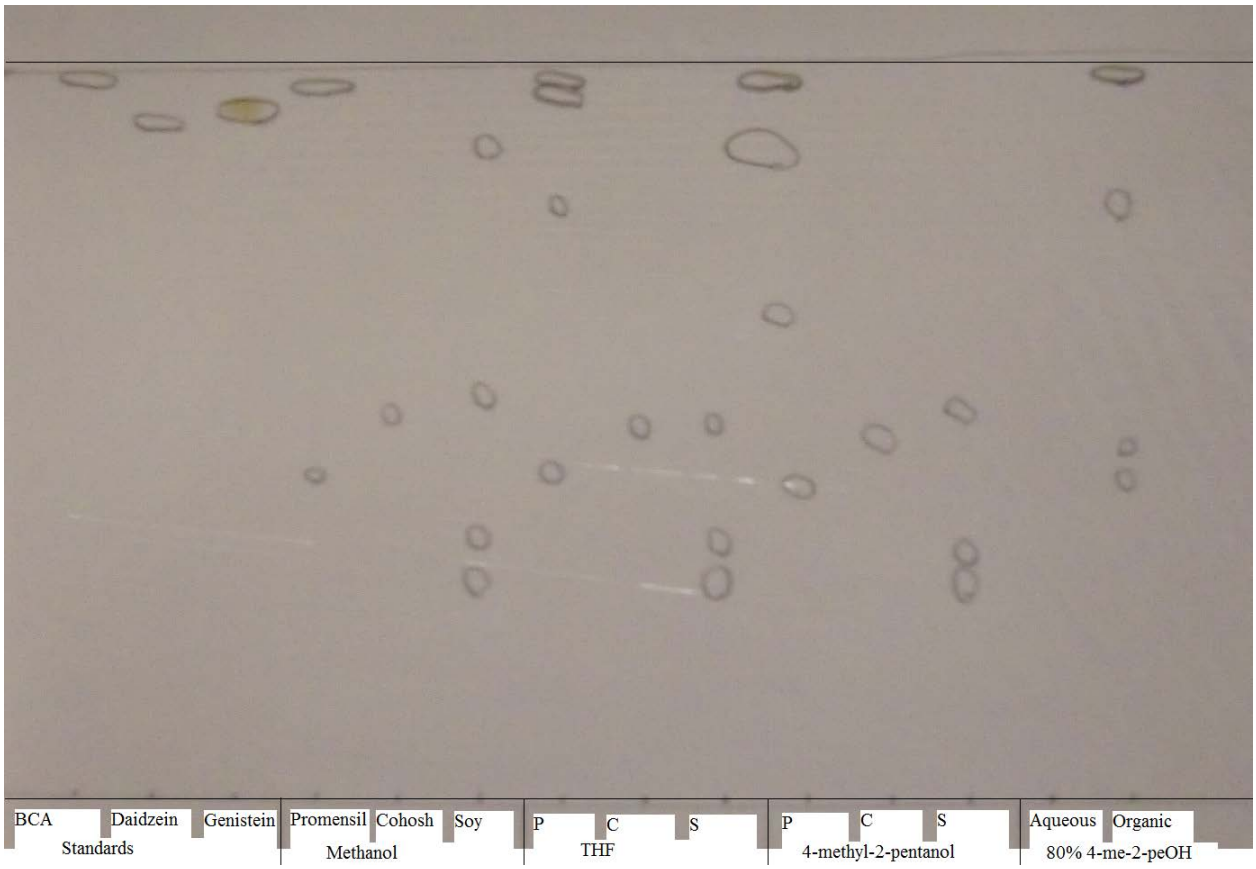


Figure 17- *Solvent test #1- 12:3:1 chloroform/methanol/acetonitrile. Decreasing the overall ratio of methanol in the solution lowers the interaction of the phytoestrogens with the TLC solvent, effectively slowing their ascent up the plate.*

In the second test (Fig. 18), the amount of acetonitrile was increased slightly as well to create a 12:3:2 chloroform/methanol/acetonitrile ratio. The results again displayed a relatively insignificant change, suggesting that a direct decrease of the amount of methanol in the mixture would be more effective than increasing the solvents with lower elutropic values.

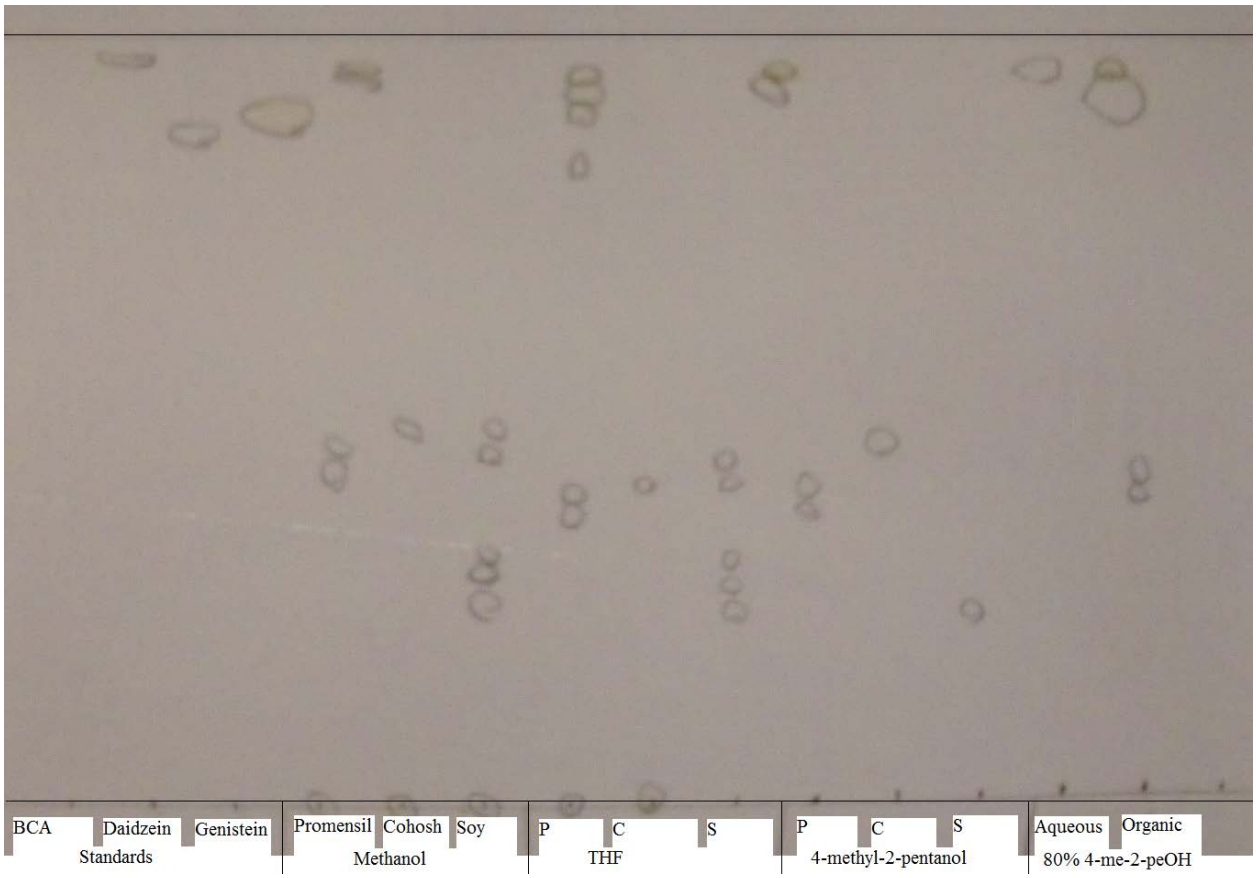


Figure 18*- Solvent test #2- 12:3:2 chloroform/methanol/acetonitrile to further lower the ratio of methanol in the solution. Biochanin A again remained with the solvent front, necessitating more modification of the solvent solution.*

To test the effects of varying amounts of methanol, three small-scale solvent tests were run using small pieces of silica with gypsum plates (Fig. 19). After viewing these results, the 12:2:1 solvent was chosen as the optimal ratio to use in the final full-scale assays.

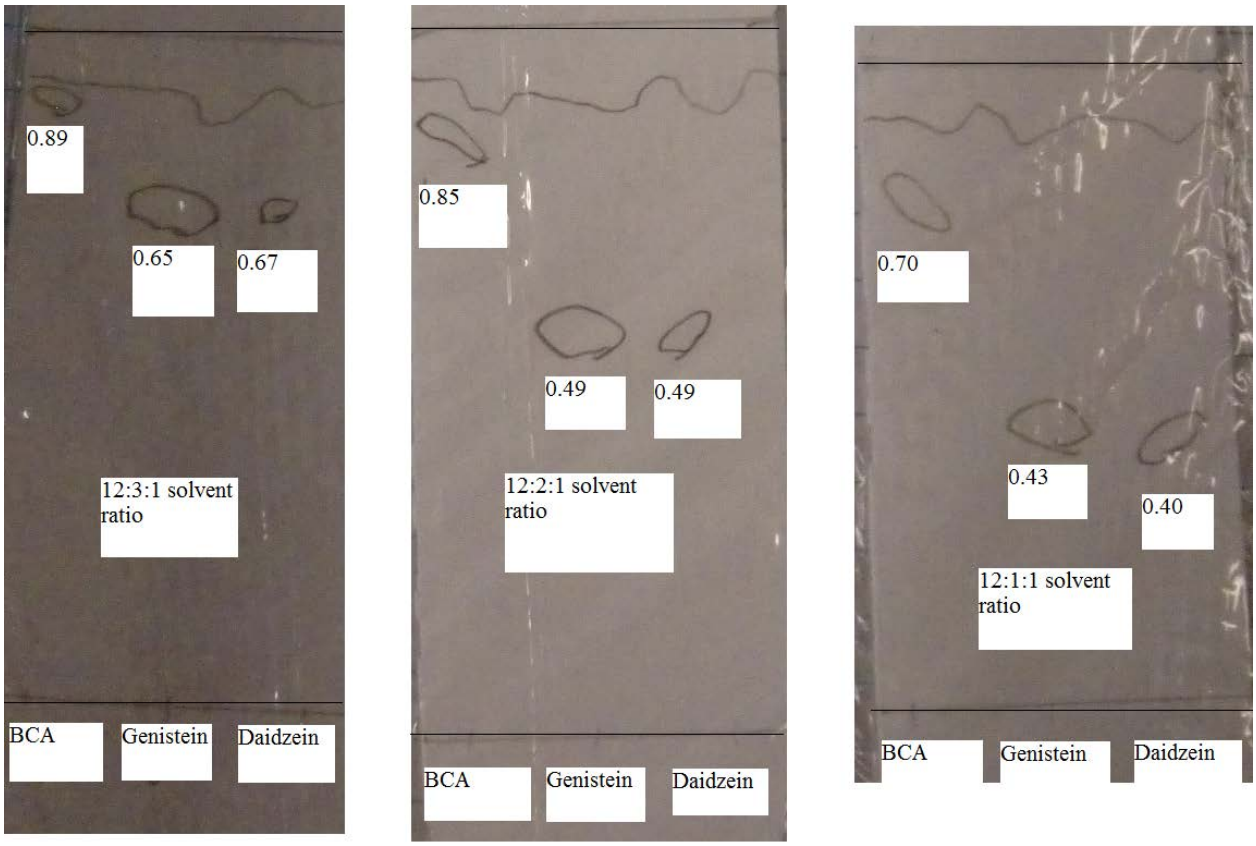


Figure 19*- Small-scale solvent tests. From left to right, solvent ratios were: 12:3:1, 12:2:1, and 12:1:1 chloroform/methanol/acetonitrile.*

The final TLC method was determined through examination of all previous tests: the 80% methanol, 80% tetrahydrofuran, and pure 4-methyl-2-pentanol extracts would be centrifuged before loading 5 µL of each supernatant onto silica F_{254} plates. For the 80% 4-methyl-2-pentanol tests, a 5 µL sample of both the aqueous and organic phases of the mixture was loaded. Purified standards of biochanin A, genistein, and daidzein were run concurrently. A solvent solution of 12:2:1 chloroform/methanol/acetonitrile was used for the mobile phase, and plates were run for 90 minutes or until the solvent front had traveled more than two-thirds of the way up the plate. Short-wave UV was used to visualize the resultant spots and calculate R_f values for their identification by comparison with the standards.

The first plate run by this method (Fig. 20) displayed a possible presence of biochanin A in each of the Promensil lanes, with bands appearing around $R_f = 0.96$ in 4 out of 5 lanes. No other bands appeared consistent with the phytoestrogen standards, although the Promensil extract using tetrahydrofuran solvent exhibited two bands, around $R_f = 0.77$ and 0.67, which were relatively close to the standard values for genistein at 0.80 and daidzein at 0.74.

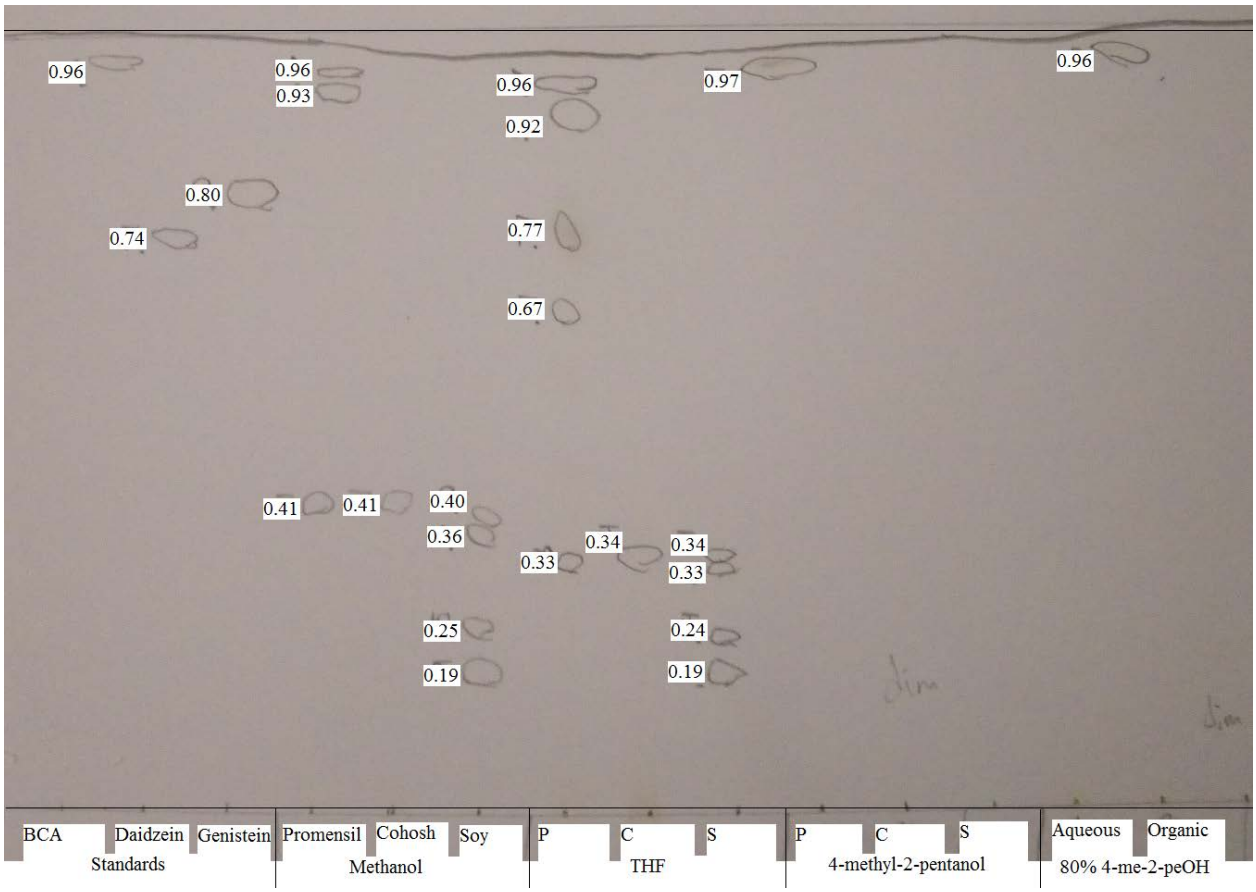


***Figure 20**- Identification of phytoestrogens in extracts, test #1. R_f values are recorded to the left of their respective bands. The presence of biochanin A is indicated by a spot near 0.96 in each extract.*

A second plate was run to check for consistency, and the results were extremely similar (Fig. 21). Most R_f values were within 0.1 or less from their counterpart bands on the first plate. Again, biochanin A was seen around R_f = 0.93 in each Promensil lane, while the two bands in the THF Promensil lane at 0.69 and 0.60 were even closer to the standard values for genistein at 0.70 and daidzein at 0.63. A compilation of these R_f values may be seen in Table 1 below.

Lane	**Test 1**	**Test 2**	**Lane**	**1**	**2**	**Lane**	**1**	**2**
Biochanin A	0.96	0.94	Daidzein	0.74	0.63	Genistein	0.80	0.70
Methanol Promensil	0.96	0.93	THF Promensil	0.67, 0.77	0.60	THF Promensil	0.77	0.69
THF Pro.	0.96	0.91						
4-methyl-2-pentanol Pro.	0.97	0.95						
80% 4M2P, Aqueous	0.96	0.94						

***Table 1**- Summary of calculated R_f values corresponding with phytoestrogen standards in Figs. 20 and 21.*

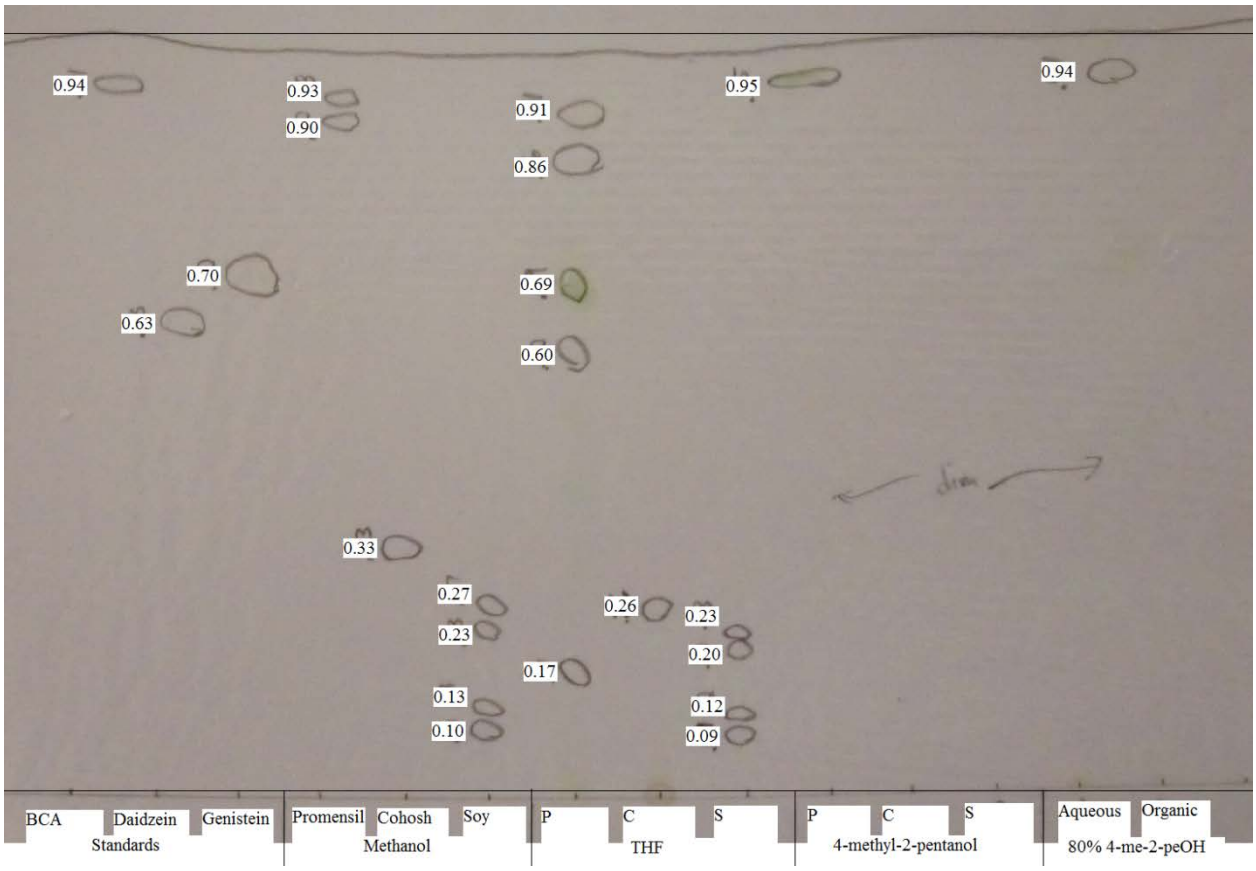


Figure 21- *Confirmation of consistency from phytoestrogen identification test #1, Fig. 20. Presence of biochanin A is observed in each extract; daidzein and genistein seem to be present in the tetrahydrofuran extract of Promensil as well.*

These results show tetrahydrofuran may be a more efficient extraction medium than methanol, at least when used for Promensil extractions. However, the bands present which match up to daidzein and genistein standards may only be pigments or other compounds which were extracted by THF and not methanol. Also, past studies using methanol extractions did display small concentrations of genistein present in Promensil extracts (Caron, 2007).

Conclusion

While the extraction of phytoestrogens using methanol has been a useful industry standard for years, it is always prudent to test alternative methods to the norm. Having a large array of techniques at one's disposal allows for more adaptability to experimental conditions as well as unforeseen circumstances. This study demonstrates a possibility for tetrahydrofuran to be used as an extraction solvent for experimentation requiring higher yields of genistein and/or daidzein. Further testing to confirm the presence of these phytoestrogens in the extract, to isolate them from the extract, and to purify them is recommended.

Despite these findings, studies such as those by Setchell *et al* and Caron still indicate methanol to be an overall effective extraction medium for over-the-counter phytoestrogen products (Setchell, 2001; Caron, 2007). When Setchell *et al* analyzed concentrations of phytoestrogens in methanol extracts of Promensil, they did find daidzein and genistein presence as well as biochanin A (Setchell, 2001). Caron's TLC method, which was the basis for this study, also displayed a presence of genistein in the methanol Promensil extract which wasn't observed in this experiment's modified method (Caron, 2007). This may be attributable to the revised method itself or to the high variability of phytoestrogen content in the over-the-counter supplements which was discussed in the introduction.

CHAPTER II: Separation of Extraction Mixtures by High-Performance Liquid Chromatography

Introduction

While TLC is a useful method to determine possible phytoestrogen content in over-the-counter supplements, it is best used in conjunction with other analyses such as high-performance liquid chromatography, or HPLC. HPLC provides not only a medium to differentiate between the components of an extraction mixture; it can also be used to analyze the relative concentrations of each component in the extraction. Coward *et al* (1993) were among the earlier groups to analyze phytoestrogens using HPLC, estimating concentrations of daidzein and genistein in high-soy diets such as those typically found in Asian countries. Setchell *et al* (2001) refined the method to be more suitable for analysis of isoflavone supplement extracts and tested 33 over-the-counter products to determine whether or not the supplements contained the amounts of phytoestrogens stated by their manufacturers. It was shown that there were many discrepancies between supplements and the authors recommended more standardization of these products should be enforced (Setchell, 2001). The Setchell *et al* method was later used by Caron (2007) and Flores *et al* (2012) to confirm phytoestrogens' presence in their supplement extracts before testing them on live cell lines.

It was briefly mentioned in the first chapter that anti-proliferative effects on cancer cells have been observed upon their exposure to certain phytoestrogens and isoflavone supplements. Caron's thesis (2007) shows a marked decrease in growth of MCF7 breast cancer cell line upon exposure to extracts from the over-the-counter supplement Promensil. Later, purified standards of phytoestrogens were tested on a T47D breast cancer cell line and it was observed that resveratrol and biochanin A were particularly effective at inducing cell apoptosis (Comeau, 2010). Further experimentation by Park and Patchel (2011) and Flores *et al* (2012) tested two other supplements, one derived from black cohosh and one from soy plants, for their effects on cell proliferation. Again Promensil displayed significant anti-proliferative qualities when tested on the MCF7 cell line; when exposed to the extract, cell counts decreased by 30-55% when compared to controls treated with only methanol. The black cohosh extract was observed to be effective as well, reducing cell growth by 35-60%. Soy extract was the weakest of the three: between the two studies it lowered cell counts anywhere from 0-35% of the control. Similar results for black cohosh and soy were obtained upon testing T47D cell lines, but oddly enough in preliminary results Promensil seemed to have little effect (Flores, 2012).

The studies by Park and Patchel (2011) and Flores et al (2012) lead one to wonder what may be the cause of the differential effects observed in their three isoflavone supplements; is it simply a matter of differing phytoestrogen concentrations or are certain phytoestrogens more effective in their anti-proliferative qualities than others? Perhaps other compounds may be

present in Promensil which amplify the anti-proliferative effects of its phytoestrogens, or it may lack certain compounds in soy and black cohosh which inhibit these effects. Whatever the case, the first step to answering these questions lies in determining the differences between the three extraction mixtures through HPLC.

Materials and Methods

Analysis by HPLC:

HPLC was carried out on a Hewlett-Packard 1100 apparatus using the method described in Setchell et al (2001) and Caron (2007). Using a PerSeptive Biosystems reverse phase PepMap C18 column, 10 µL of sample or standard were injected at a flow rate of 1.0 mL/min. The column was then re-equilibrated in 10 mM ammonium acetate (0.1% trifluoroacetic acid [TFA]) for 2 minutes. Elution followed for 22 minutes using a linear gradient of 10 mM ammonium acetate (0.1% TFA) 100-50% and acetonitrile 0-50% until the column was held isocratic at 50/50 for 5 minutes. The column was then re-equilibrated using 10 mM ammonium acetate (0.1% TFA). Absorbance was measured at 260 nm for the entire process. 100 µM pure standards of genistein, daidzein, and biochanin A were prepared in methanol and run separately to determine their respective retention times. The extraction mixtures were centrifuged at 3000 RPM for 5 minutes and the supernatant passed through a 0.8 µm syringe filter to eliminate particulates before being run.

As detailed in Chapter I, supplement extractions were carried out by refluxing each of Promensil, soy, and black cohosh supplements in 80% methanol for one hour. The resulting mixtures were filtered and used as the three HPLC extract samples.

Hewlett-Packard ChemStation software for liquid chromatography systems was used for data analysis and determining integration values of HPLC traces.

Results and Discussion

From this point on, HPLC data will be presented and discussed using only specific traces selected from three runs per each of three phytoestrogen standards and three extract samples. To see the full set of traces as well as integration data for each, please refer to the Appendix.

The three pure phytoestrogen standards were each run in triplicate to confirm consistency of the observed peak and calculate average retention times for each standard. These results may be seen in Table 2 below.

Standard	Run 1 RT (min.)	Run 2 RT (min.)	Run 3 RT (min.)	% Variance	Avg. RT (min.)
Daidzein	20.061	20.120	20.106	0.29	20.096
Genistein	22.956	23.060	22.965	0.45	22.994
Biochanin A	28.279	28.310	28.220	0.32	28.270

***Table 2**-Retention times of three phytoestrogen standards. The standards were prepared to 100 µM in methanol. Percent variance between the highest and lowest RT for each standard was calculated to confirm consistency.*

All standards had a variance of less than 0.5% among three runs, confirming precision of the method and apparatus used. For later comparison to the phytoestrogen extracts average retention times were calculated to be 20.096 min. for daidzein, 22.994 for genistein, and 28.270 for biochanin A. Expected retention times for daidzein, genistein, and biochanin A were derived from Setchell (2001), Caron (2007), and Flores (2012). These were estimated at 21, 23, and 29 minutes respectively. Thus the observed average retention times were earlier than expected, but consistently so; this can be attributed to the use of a different apparatus or HPLC column than the previous experiments. Due to the low calculated percent error the difference is negligible.

Upon examination of the Promensil extract traces, the peaks with retention times most comparable to the standards were found at 20.179, 23.049, and 28.255 minutes respectively. It is assumed these peaks represent daidzein, genistein, and biochanin A presence. The biochanin A peak was especially pronounced. Taking the results of Comeau and Skorinko into consideration, it is likely that biochanin A plays a large part in the observed effectiveness of Promensil in reducing cell proliferation (Comeau, 2010). An unknown major peak was also observed at 24.910 minutes. Other peaks of interest, whose percent areas after integration were greater than one, occurred at retention times of 19.166, 20.910, 21.649, 22.160, 23.680, and 26.794 minutes. These results may be seen in Figure 22 below.

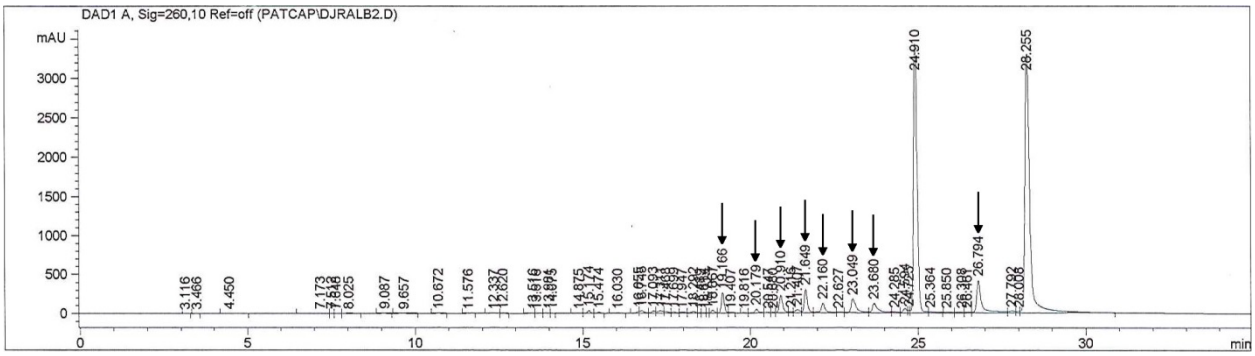


Figure 22 – *HPLC trace of Promensil extract. Major peaks are seen at retention times of 24.910 and 28.255 minutes. Arrows indicate peaks of interest with areas greater than one percent.*

To confirm the identity of the three possible phytoestrogen peaks, a second Promensil sample was spiked with the pure phytoestrogen standards and run through HPLC. This result may be seen below (Fig. 23).

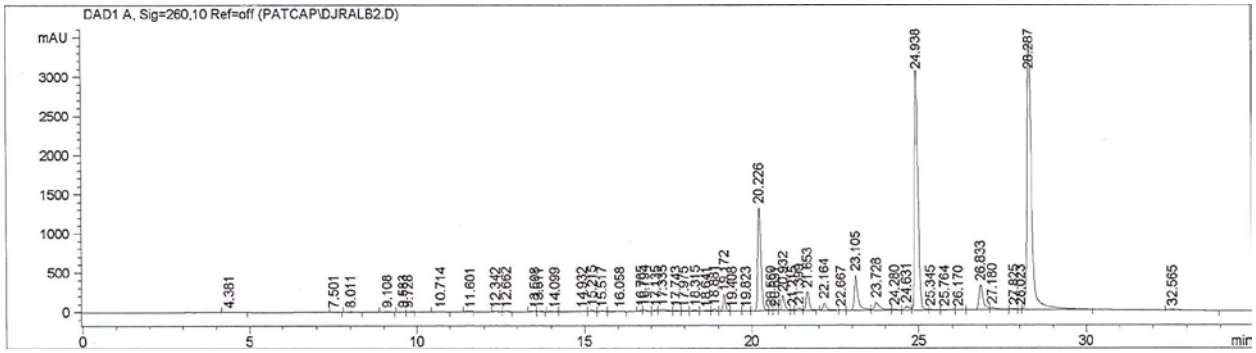


Figure 23 – *HPLC trace of Promensil extract spiked with pure standards of daidzein, genistein, and biochanin A. The three peaks of interest from the base Promensil extract were analyzed, and two of three displayed an increased % area over the initial Promensil runs. The peak at 28.287, corresponding with biochanin A, was off the scale and thus no difference could be observed.*

Average retention times and percent areas of the relevant peaks in both the initial Promensil extract runs and the spiked runs were calculated for comparison between the two. Results displayed a ratio greater than one between percent areas of the spiked and unspiked mixtures for both peaks representing daidzein and genistein near 20.096 and 22.994 respectively; this data as well as there being no change in the peaks' shape confirms the identity and presence of daidzein and genistein in the Promensil extract. The peak at 28.287 of the spiked mixture exhibited a similar percent area to the corresponding peak on the initial Promensil run rather than increasing, but this discrepancy may be ignored since both signals were too strong for the detector. There was no change in the shape of this peak so it will be assumed it represents a high concentration of biochanin A. This corresponds with the findings of Setchell *et al*: their test of Promensil displayed a high concentration of biochanin A in the extract as well (Setchell, 2001). For further confirmation one could dilute the Promensil extract to weaken the signal at 28.287 then spike the diluted mixture with pure biochanin A standard, but this was deemed unnecessary for this study

after considering the results of Setchell *et al*. Data from comparison of the initial Promensil extract and the spiked extract may be seen in Table 3 below.

Compound	Avg. Standard RT (min.)	Nearest Peak RT (min.)	Peak % Area	Spiked Nearest Peak RT (min.)	Spiked Peak % Area	Area Ratio (spiked/ unspiked)
Daidzein	20.096	20.171	0.4839	20.226	9.9903	20.65
Genistein	22.994	23.039	2.4614	23.108	4.7160	1.916
Biochanin A	28.270	28.243	41.928	28.289	40.948	0.9766

Table 3 – *Analysis of HPLC data between initial Promensil extract runs and Promensil spiked with phytoestrogen standards. Ratios increased when the spiked peak percent areas of daidzein and genistein peaks were compared to the corresponding initial Promensil extract peak percent areas, confirming the identity of those peaks.*

The soy extract (Fig. 24) exhibited exceedingly small peaks at the three phytoestrogen standard retention times. One peak at 20.168 minutes may coincide with daidzein; genistein and biochanin A peak areas were so small as to be negligible when compared to other components of the mixture. Larger, unidentified peaks were observed at 15.174, 15.475, 17.084, 18.242, and 18.474. Other notable peaks occurred at 14.912, 20.305, and 20.528 minutes.

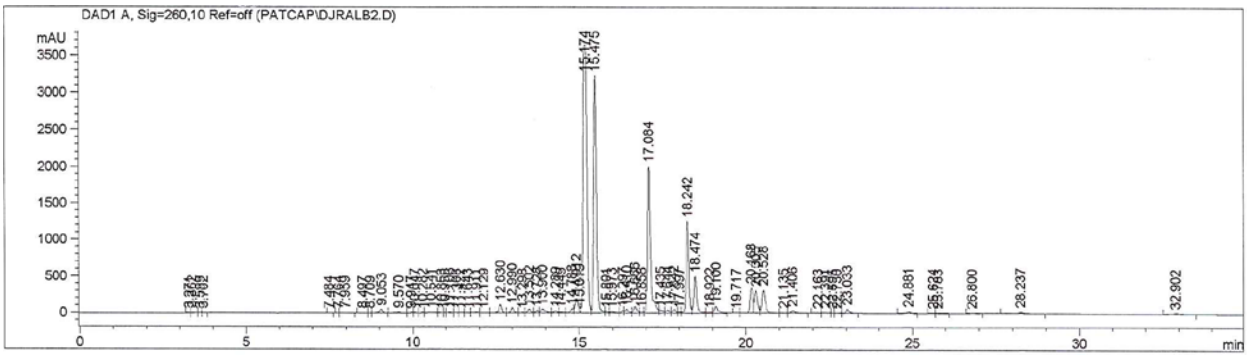


Figure 24 – *HPLC trace of soy extract. Several major peaks were observed; none coincided with phytoestrogen standard RTs.*

Black cohosh extract (Fig. 25) yielded a relatively large peak at 20.096 minutes, coinciding exactly with the calculated retention time for daidzein. A small peak at 28.243 displays possible biochanin A content as well; as with Promensil, this may contribute to the black cohosh extract's observed anti-proliferative effects in previous tests (Park, 2011; Flores, 2012). One small peak at 23.188 suggests low genistein concentrations. Major peaks were also observed at 17.074 and 21.388 minutes. Other peaks of interest occurred at 7.996, 8.587, 9.018, 9.530, 10.177, 10.274, 10.689, 11.478, 12.039, 12.670, 13.523, 14.001, 15.045, 16.574, 17.419, 17.665, 18.057, 18.972, 19.863, 22.386, 26.950, 27.593, and 28.515 minutes. The high incidence of this many peaks displaying areas greater than one percent suggests the anti-

proliferative effects of the black cohosh mixture may be attributable not only to its phytoestrogen content, but also to an efficient combination of many constituents. Results may be seen below.

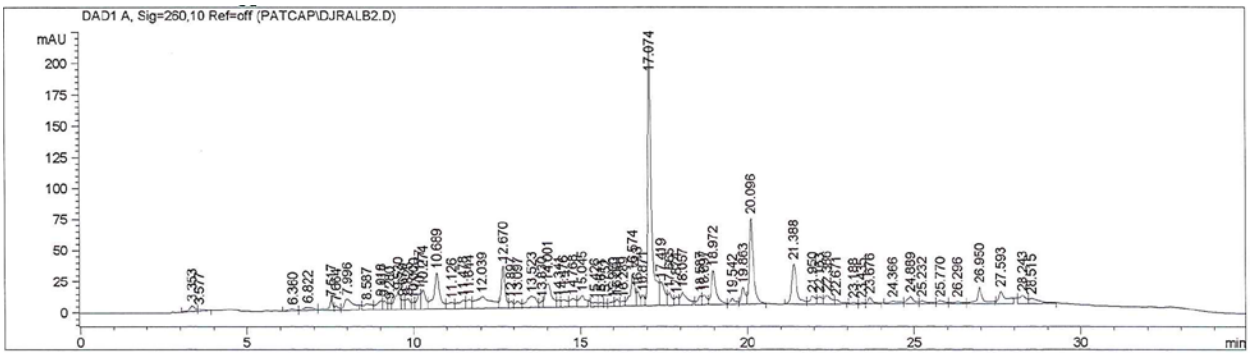


Figure 25 –*HPLC trace of black cohosh extract. The major peak at 20.096 coincides exactly with the calculated average retention time for daidzein.*

In a cursory visual comparison of the three extracts it quickly becomes apparent there are significant differences in retention times between their major peaks. One's eye is drawn to the peaks occurring at 24.910 and 28.255 for Promensil; at 17.074, 20.096, and 21.388 for black cohosh; and at 15.174, 15.475, 17.084, 18.242, and 18.474 for soy. Even without referencing the integration data the height of these peaks causes them to stand out among the traces. Closer examination of integration data involving all peaks of interest shows several unique peaks in each extract. The major peak at 24.910 of the Promensil extract stands out in particular with a strong reading that exceeds the scale of the trace, but peaks of interest at 19.166, 20.910, 23.680, and 26.794 are also unique to Promensil.

The soy extract was different in that all its peaks of interest occurred before 21 minutes, effectively ruling out genistein at 22.944 and biochanin A at 28.270. The presence of daidzein was likely with a peak observed at 20.168. One of soy's major peaks at retention time 17.084 coincided with a strong black cohosh peak at 17.074. Considering the large disparity between the anti-proliferative effects of soy and black cohosh (Park, 2011; Flores, 2012), it is possible to rule out these peaks as having little to no influence on the anti-proliferative effects of the extracts as a whole. The remaining peaks of interest unique to soy occur at 14.912, 15.174, 15.475, 18.242, 18.474, 20.305, and 20.528 minutes.

Black cohosh displayed more than twice as many peaks with areas greater than one percent when compared to the other two extracts. The three strongest signals were measured at 17.074, 20.096, and 21.388. As mentioned in the previous paragraph, the peak at 17.074 coincides with a large peak in soy and is therefore ruled out of the equation when considering the differential effects between these mixtures. The peak at 20.096 coincides precisely with the calculated retention time for the pure daidzein standard. Of particular interest is the peak

occurring at 21.388, which is unique to the black cohosh extract. Since the peaks of interest were so numerous in the black cohosh extract, there are myriad others which were unique to the mixture; those occurring at 10.689, 12.670, and 18.972 demonstrated the strongest signals of the remaining peaks.

The assumed common presence of biochanin A in both Promensil and black cohosh suggests the compound may have the strongest anti-proliferative effect of the three phytoestrogen standards tested since both extracts exhibited such strong effects on live cell lines (Park, 2011; Flores, 2012). The soy extract exhibited a negligible peak at the corresponding retention time, possibly accounting for it having the weakest effect from among the three supplements.

Possible presence of daidzein was observed in varying concentrations in all three extracts. This indicates a possibility that any anti-proliferative effects of daidzein may be highly dependent on concentration, as the lowest concentration occurred in the relatively ineffective soy product. Another possibility is that daidzein works more efficiently in tandem with biochanin A, which is absent from the soy extract.

Conclusion

The intent of this second chapter was to determine unique components between the three phytoestrogen supplement extracts which would help to explain the differences in their effects on breast cancer cell proliferation. While there were several constituents unique to each mixture, those present in the soy extract were somewhat less intriguing due to its weak observed anti-proliferative effects (Park, 2011; Flores, 2012). That being said, unless more positive results are found for the soy extract in the future it may not be a worthwhile pursuit for anti-proliferation testing. Phytoestrogen content in the soy was also very low among the three standards run for comparison, although this may simply be a result of the product being unregulated. The results of Coward *et al* reinforce this fact; natural intake of soy in one's diet would seem to be more effective than this soy supplement in certain instances (Coward, 1993). Perhaps there is more to the soy diet's success than its phytoestrogen content.

The significant anti-proliferative effects of the Promensil and black cohosh extracts may be attributable to their combinations of phytoestrogens, but both extracts exhibited their own strong unique peaks as well which may be contributing factors in their effectiveness. Promensil seems to contain a larger concentration of biochanin A, while black cohosh displayed more daidzein. Tests on breast cancer cell lines involving combinations of daidzein and biochanin A in various ratios would be helpful to determine whether or not these compounds have a greater anti-proliferative effect in tandem than they do alone. Isolation and analysis of the major Promensil peaks at 24.910 and 28.255 is strongly recommended for further testing on live cell lines, as well as the major black cohosh peak at 21.388.

Setchell *et al* found biochanin A to be the largest phytoestrogen component of Promensil by far (44,330 µg/g), which helps explain why the signal strength in this study's extract was far too strong for the HPLC spectrometer to measure correctly. Also occurring in a high concentration was the isoflavone formononetin at a concentration of 26,726 µg/g; this may account for the large unknown peak on the Promensil trace at 24.910. Daidzein content was calculated at 1,532 µg/g and genistein at 2,900 µg/g (Setchell, 2001). Here the variable nature of these over-the-counter supplements may be seen again; the HPLC signals observed from this study's Promensil extract indicated a slightly higher concentration of daidzein rather than genistein in the extract.

Another point to take into consideration when discussing the anti-proliferative effects of phytoestrogens is their possible metabolism when introduced into the human body. Due to their structural similarities to human estrogens it's entirely possible phytoestrogens may be broken down into metabolite forms as well. This may explain the differential effects observed in natural soy diets over soy isoflavone supplements due to the difference in methods of delivery (Coward, 1993). While the compounds themselves seem to have effects on cancer cell proliferation when

introduced directly to cell lines via petri dishes, it would seem to be more difficult for them to stay intact and reach their desired destination in the body when they must be ingested and first travel through the stomach to the bloodstream to be distributed. With the high likelihood of some sort of metabolism taking place along the way, phytoestrogens from supplements would not arrive at the breast epithelium in a pure form but instead in a mixture of metabolites which may induce different effects. Future studies could be developed on this basis; perhaps phytoestrogen standards could be treated with estrogen-metabolizing enzymes and then added to cancer cell lines to observe any differential effects from the pure compounds.

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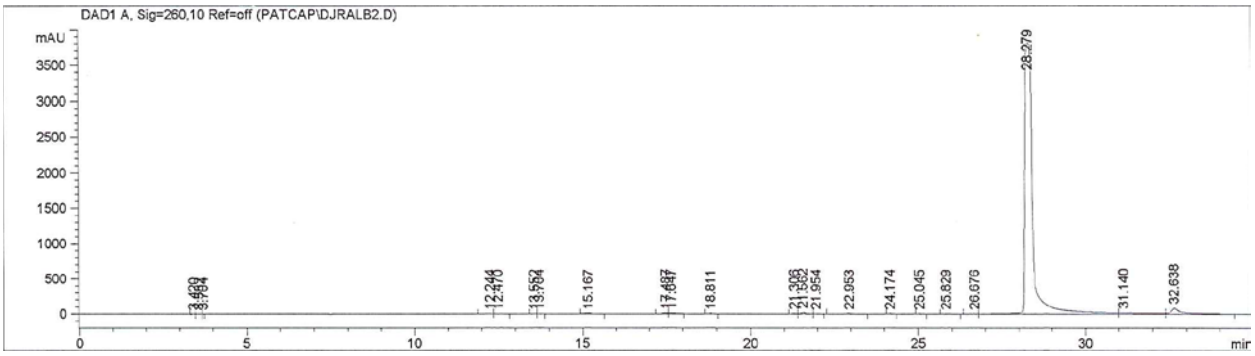
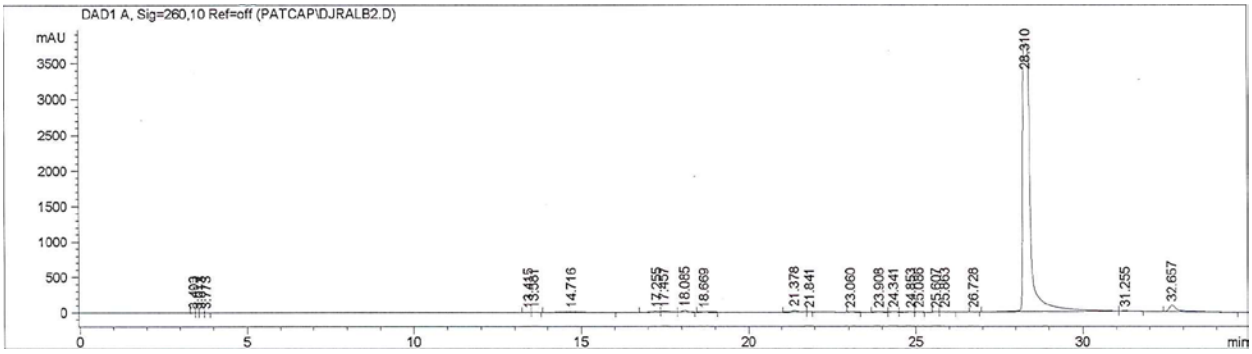
Appendix of HPLC Data

Biochanin A: 1



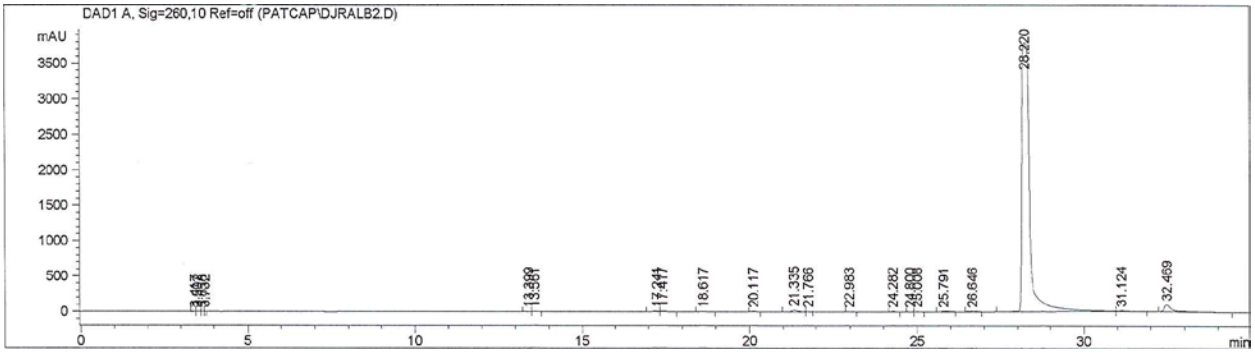
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.420	PV	0.0634	11.20100	2.44862	0.0170
2	3.582	VV	0.1414	18.06853	1.70771	0.0274
3	3.704	VV	0.0699	6.17896	1.28871	9.366e-3
4	12.244	PV	0.2205	21.71588	1.38819	0.0329
5	12.470	VB	0.2153	19.59199	1.38257	0.0297
6	13.552	BV	0.1180	12.11399	1.57050	0.0184
7	13.704	VB	0.1186	10.64493	1.31292	0.0161
8	15.167	BP	0.2180	12.55112	1.01661	0.0190
9	17.487	BV	0.1729	115.85877	10.17288	0.1756
10	17.647	VB	0.1746	113.15131	9.00596	0.1715
11	18.811	BP	0.1289	30.11488	3.62577	0.0456
12	21.306	BV	0.1332	79.45633	8.98265	0.1204
13	21.562	VV	0.1783	225.95802	18.78490	0.3425
14	21.954	VB	0.2092	43.78877	2.81921	0.0664
15	22.953	BB	0.4183	165.70235	4.95290	0.2512
16	24.174	PP	0.0978	27.99469	4.42661	0.0424
17	25.045	VP	0.1066	27.27678	3.85132	0.0413
18	25.829	BB	0.2245	60.51794	3.55601	0.0917
19	26.676	BV	0.2157	48.11082	2.95885	0.0729
20	28.279	VV	0.2510	6.17091e4	3808.43311	93.5333
21	31.140	VV	0.7552	1162.33191	18.75120	1.7618
22	32.638	VB	0.3350	2054.09790	82.90932	3.1134

Biochanin A: 2



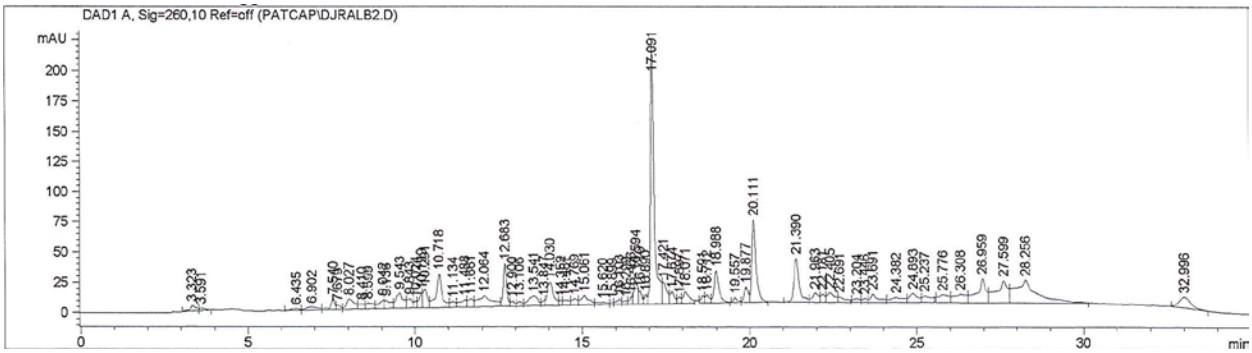
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.403	PV	0.0880	18.07241	3.01090	0.0278
2	3.517	VV	0.0843	14.98076	2.41641	0.0230
3	3.613	VV	0.1021	14.04636	1.85992	0.0216
4	3.773	VB	0.0987	9.01611	1.30325	0.0139
5	13.415	BV	0.1388	16.45853	1.79722	0.0253
6	13.581	VB	0.1603	18.77824	1.58418	0.0289
7	14.716	BB	0.6954	351.55414	6.41434	0.5402
8	17.255	BV	0.2597	223.31731	12.78981	0.3432
9	17.457	VV	0.2783	267.37350	12.77113	0.4109
10	18.085	VB	0.1696	315.82928	27.58400	0.4853
11	18.669	BB	0.2409	48.16734	2.97457	0.0740
12	21.378	PB	0.1943	298.60202	24.47640	0.4588
13	21.841	BV	0.1131	8.24754	1.10448	0.0127
14	23.060	BB	0.1310	17.67395	1.92648	0.0272
15	23.908	BP	0.2630	16.79512	1.01528	0.0258
16	24.341	VB	0.1013	36.13482	5.45036	0.0555
17	24.853	BV	0.1708	18.50546	1.47080	0.0284
18	25.086	VP	0.0990	23.28753	3.62461	0.0358
19	25.607	PV	0.0962	9.64060	1.55880	0.0148
20	25.863	VP	0.1749	45.92624	3.85858	0.0706
21	26.728	BB	0.1450	19.95387	1.92359	0.0307
22	28.310	BV	0.2516	6.13781e4	3776.39771	94.3159
23	31.255	VB	0.2738	208.63383	10.41256	0.3206
24	32.657	BP	0.2628	1698.05786	90.52945	2.6093

Biochanin A: 3



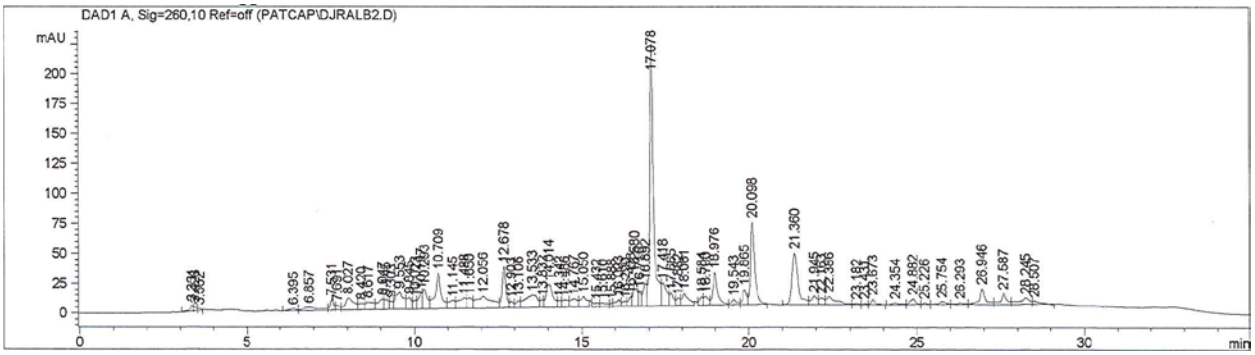
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.417	PV	0.0888	20.39213	3.35997	0.0320
2	3.502	VV	0.0995	20.93274	2.79210	0.0329
3	3.658	VV	0.0744	10.52995	2.03288	0.0165
4	3.732	VV	0.0539	5.68595	1.51135	8.929e-3
5	13.399	BV	0.1334	13.01288	1.49660	0.0204
6	13.561	VB	0.1275	11.77038	1.32616	0.0185
7	17.241	BV	0.2005	109.33137	8.58887	0.1717
8	17.417	VB	0.1918	102.68456	7.49797	0.1612
9	18.617	PB	0.2025	31.64322	2.48549	0.0497
10	20.117	BB	0.1193	50.81451	6.49377	0.0798
11	21.335	PB	0.1993	297.13226	23.53485	0.4666
12	21.766	BV	0.1237	12.76559	1.55572	0.0200
13	22.983	PB	0.1204	16.37828	2.02504	0.0257
14	24.282	PB	0.0997	33.27325	5.13012	0.0523
15	24.800	PV	0.1025	7.95121	1.18216	0.0125
16	25.008	VP	0.1049	24.83398	3.67128	0.0390
17	25.791	BB	0.1871	47.93239	3.80219	0.0753
18	26.646	PB	0.1527	22.39539	2.06245	0.0352
19	28.220	BV	0.2493	6.08508e4	3790.10913	95.5566
20	31.124	VB	0.3156	287.58640	12.17243	0.4516
21	32.469	BP	0.2473	1702.52307	97.69281	2.6735

Black Cohosh: 1



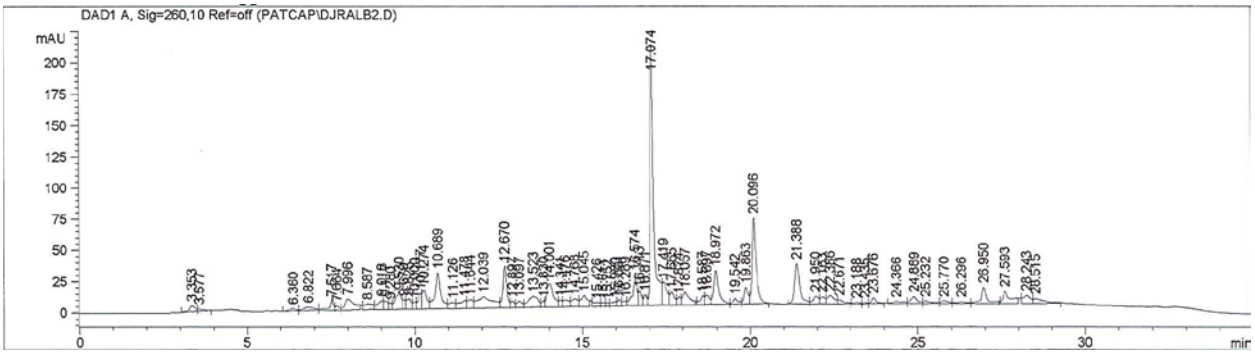
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.323	BV	0.1700	60.98908	4.56121	0.5808
2	3.591	VB	0.1490	24.04328	2.07776	0.2290
3	6.435	BV	0.2337	27.37380	1.64882	0.2607
4	6.902	VV	0.3858	62.90607	2.49791	0.5991
5	7.540	VV	0.1226	82.68661	9.59315	0.7874
6	7.679	VV	0.1112	31.76017	3.89241	0.3025
7	8.027	VV	0.2489	147.64920	8.66054	1.4061
8	8.410	VV	0.1609	53.76667	4.45302	0.5120
9	8.599	VV	0.2039	81.88670	5.25236	0.7798
10	9.042	VV	0.1550	89.44131	7.50674	0.8518
11	9.136	VV	0.1650	91.98546	7.20040	0.8760
12	9.543	VV	0.2165	210.65985	12.90072	2.0061
13	9.843	VV	0.1361	82.19699	8.11950	0.7828
14	10.024	VV	0.0974	41.65988	6.12066	0.3967
15	10.199	VV	0.0993	90.09926	12.61869	0.8580
16	10.291	VV	0.1313	148.40932	15.84051	1.4133
17	10.718	VB	0.1753	360.27463	27.77673	3.4309
18	11.134	BV	0.1520	53.85292	5.06866	0.5128
19	11.488	VV	0.2053	97.92434	6.23411	0.9325
20	11.661	VV	0.1554	79.87265	7.09228	0.7606
21	12.064	VV	0.3909	281.89700	9.16361	2.6845
22	12.683	VV	0.1048	249.56459	35.15208	2.3766
23	12.900	VV	0.1174	37.89929	4.63393	0.3609
24	13.106	VV	0.1492	45.37468	4.16294	0.4321
25	13.541	VV	0.2536	170.79237	8.38467	1.6265
26	13.847	VV	0.0984	34.79358	5.04877	0.3313
27	14.030	VV	0.1941	250.44551	19.20661	2.3850
28	14.352	VV	0.1020	33.51738	4.65788	0.3192
29	14.487	VV	0.1736	60.58190	4.78929	0.5769
30	14.769	VV	0.1732	71.00723	5.62852	0.6762
31	15.061	VV	0.2246	138.66101	7.98320	1.3205
32	15.620	VV	0.2770	51.35415	2.36833	0.4890
33	15.899	VV	0.0848	12.20298	2.13254	0.1162
34	16.103	VV	0.1328	47.03699	4.78119	0.4479
35	16.299	VV	0.1362	27.88098	3.12060	0.2655
36	16.466	VV	0.0922	43.60940	6.85850	0.4153
37	16.594	VV	0.1024	171.61555	24.90004	1.6343
38	16.719	VV	0.0894	65.43214	11.00636	0.6231
39	16.890	VV	0.0938	50.52049	7.98174	0.4811
40	17.091	VV	0.1076	1571.53760	214.17270	14.9659
41	17.421	VV	0.1425	178.87227	17.91049	1.7034
42	17.674	VV	0.1240	88.24178	9.90363	0.8403
43	17.897	VV	0.1109	43.30822	5.43672	0.4124
44	18.071	VB	0.1732	128.55324	9.78817	1.2242
45	18.591	BV	0.0956	42.61738	6.40425	0.4058
46	18.712	VV	0.1286	64.19851	7.02857	0.6114
47	18.988	VB	0.1407	265.61191	26.55923	2.5294
48	19.557	BV	0.1322	39.56938	4.26759	0.3768
49	19.877	VV	0.1202	106.46107	12.91191	1.0138
50	20.111	VB	0.1245	584.93427	67.93098	5.5704
51	21.390	PV	0.1703	413.51175	35.92213	3.9379
52	21.963	VV	0.1776	100.52322	7.73819	0.9573
53	22.181	VV	0.1428	64.26675	6.41991	0.6120
54	22.405	VV	0.2189	126.00420	8.03853	1.1999
55	22.691	VB	0.2102	70.43025	4.50970	0.6707
56	23.204	BV	0.2114	45.83569	2.98038	0.4365
57	23.446	VV	0.1502	33.82428	3.17696	0.3221
58	23.691	VB	0.2342	120.56513	6.88844	1.1482
59	24.382	BV	0.3835	123.51821	4.27018	1.1763
60	24.893	VV	0.2447	141.53395	7.76190	1.3478
61	25.237	VB	0.2594	110.58450	5.48012	1.0531
62	25.776	BV	0.3054	151.48291	6.61028	1.4426
63	26.308	VV	0.3505	194.31020	6.98534	1.8504
64	26.959	VV	0.2420	370.72256	20.02157	3.5304
65	27.599	VV	0.3086	440.76840	18.19601	4.1975
66	28.256	VB	0.5832	892.04681	18.87951	8.4950
67	32.996	BB	0.3706	223.34970	9.02811	2.1270

Black Cohosh: 2



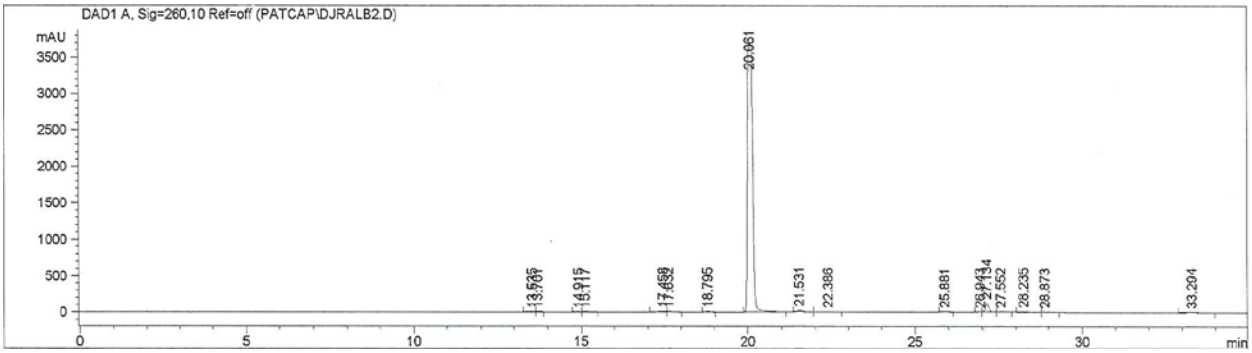
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.331	BV	0.1237	39.30570	4.69513	0.4122
2	3.428	VV	0.0791	20.64158	3.69832	0.2164
3	3.562	VV	0.0948	12.42051	1.75054	0.1302
4	6.395	BV	0.2464	28.55786	1.75012	0.2995
5	6.857	VB	0.4750	94.78476	2.82786	0.9939
6	7.531	BV	0.1076	64.50724	9.00755	0.6764
7	7.691	VV	0.1226	36.76585	4.10122	0.3855
8	8.027	VV	0.2657	185.98834	10.05992	1.9503
9	8.420	VV	0.1552	62.71326	5.41073	0.6576
10	8.617	VV	0.2177	107.87637	6.43199	1.1312
11	9.047	VV	0.1672	117.29130	9.05272	1.2299
12	9.145	VV	0.1103	70.78458	8.76157	0.7423
13	9.303	VV	0.0937	46.35076	6.95603	0.4860
14	9.553	VV	0.2260	248.41360	14.48875	2.6049
15	9.845	VV	0.1401	102.97150	10.00381	1.0798
16	10.023	VV	0.0972	51.70028	7.61819	0.5421
17	10.197	VV	0.1055	106.50985	14.20583	1.1169
18	10.293	VV	0.1377	167.62688	17.19443	1.7577
19	10.709	VV	0.1780	393.66776	30.20677	4.1280
20	11.145	VV	0.1910	97.77959	7.08767	1.0253
21	11.488	VV	0.2206	151.97136	8.92864	1.5936
22	11.650	VV	0.1484	95.85345	8.84721	1.0051
23	12.056	VV	0.4088	333.12244	10.15201	3.4932
24	12.678	VV	0.1130	266.59232	34.94928	2.7955
25	12.903	VV	0.1217	48.63943	5.69382	0.5100
26	13.106	VV	0.1249	54.14741	6.02239	0.5678
27	13.533	VV	0.3132	283.22906	11.11851	2.9700
28	13.832	VV	0.0994	47.06667	6.74471	0.4935
29	14.014	VV	0.2095	279.42154	19.69484	2.9300
30	14.342	VV	0.1064	43.98084	5.93851	0.4612
31	14.486	VV	0.1774	79.43365	6.04171	0.8329
32	14.767	VV	0.1912	99.47188	7.10946	1.0431
33	15.050	VB	0.1878	128.43883	9.03874	1.3468
34	15.432	BV	0.1844	47.37115	3.36102	0.4967
35	15.610	VV	0.1870	45.43488	3.25157	0.4764
36	15.888	VV	0.0878	19.28909	3.22361	0.2023
37	16.093	VV	0.1446	61.74771	5.69234	0.6475
38	16.290	VV	0.1424	40.06229	4.31005	0.4201
39	16.458	VV	0.0956	53.86622	8.09390	0.5648
40	16.580	VV	0.1041	182.98045	26.01213	1.9187
41	16.716	VV	0.0909	81.44489	13.02529	0.8540
42	16.892	VV	0.1088	143.91362	19.35254	1.5091
43	17.078	VV	0.1122	1695.89709	219.34052	17.7833
44	17.418	VV	0.1510	207.11124	19.33635	2.1718
45	17.665	VV	0.1220	96.19509	11.00613	1.0087
46	17.882	VV	0.1110	51.56202	6.46776	0.5407
47	18.061	VB	0.1868	149.84564	10.61003	1.5713
48	18.584	BV	0.1018	53.30073	7.41695	0.5589
49	18.700	VV	0.1303	73.94314	7.96646	0.7754
50	18.976	VB	0.1447	286.19873	27.65849	3.0011
51	19.543	BV	0.1460	49.26227	4.70937	0.5166
52	19.865	VV	0.1211	111.98000	13.45127	1.1742
53	20.098	VB	0.1242	592.30304	68.93871	6.2109
54	21.360	PB	0.1925	551.45453	43.31725	5.7826
55	21.945	BV	0.1718	95.34554	7.62968	0.9998
56	22.163	VV	0.1396	55.57648	5.70976	0.5828
57	22.386	VB	0.2725	144.10449	7.04849	1.5111
58	23.182	BV	0.1690	15.03723	1.29949	0.1577
59	23.431	VV	0.1145	8.85270	1.16674	0.0928
60	23.673	VB	0.1298	40.70549	4.66391	0.4268
61	24.354	PV	0.2456	27.16561	1.63746	0.2849
62	24.882	VV	0.1617	56.06490	4.82029	0.5879
63	25.226	VV	0.1275	14.73017	1.65978	0.1545
64	25.754	VV	0.2279	42.54084	2.64097	0.4461
65	26.293	VV	0.2122	26.62823	1.66824	0.2792
66	26.946	VV	0.1674	164.07671	13.54009	1.7205
67	27.587	VV	0.1724	120.19996	9.58078	1.2604
68	28.245	VV	0.2713	116.07616	5.61247	1.2172
69	28.507	VP	0.2112	46.15210	2.90675	0.4840

Black Cohosh: 3



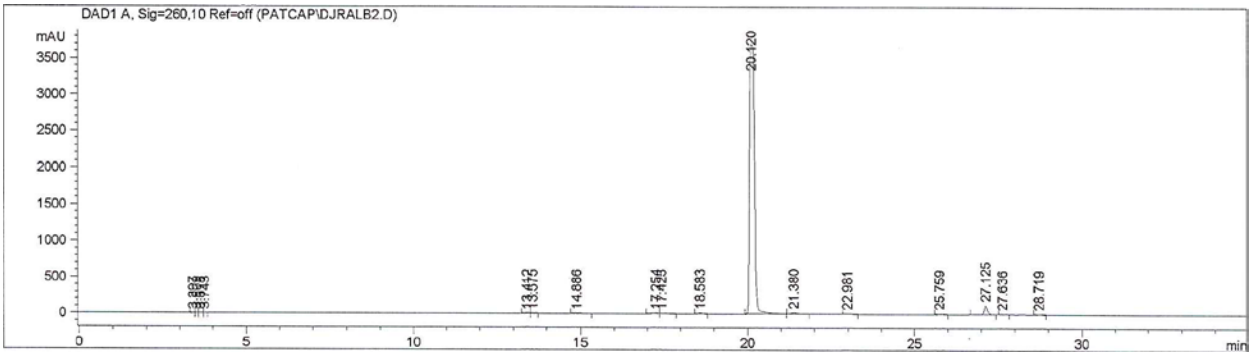
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.353	BV	0.1732	59.42638	4.77480	0.6885
2	3.577	VB	0.1838	24.28883	1.79554	0.2814
3	6.360	BV	0.2386	28.93984	1.86978	0.3353
4	6.822	VV	0.3854	69.45670	2.74352	0.8047
5	7.517	VV	0.1366	77.64898	7.77016	0.8996
6	7.664	VV	0.1151	31.44668	3.77811	0.3643
7	7.996	VB	0.2836	180.60890	8.94316	2.0924
8	8.587	BV	0.2334	88.01527	4.85674	1.0197
9	9.018	VV	0.1558	91.22655	7.50453	1.0569
10	9.117	VV	0.1081	56.07507	7.10027	0.6497
11	9.280	VV	0.0949	34.33986	5.21068	0.3978
12	9.530	VV	0.1825	165.85042	12.52522	1.9215
13	9.670	VV	0.0790	41.10794	7.60760	0.4763
14	9.825	VV	0.1381	84.79151	8.37572	0.9823
15	10.000	VV	0.0964	39.97041	5.94949	0.4631
16	10.177	VV	0.1048	91.29635	12.55384	1.0577
17	10.274	VV	0.1337	145.23837	15.44192	1.6827
18	10.689	VB	0.1669	356.15793	29.07037	4.1263
19	11.126	BV	0.1835	66.72641	5.07073	0.7731
20	11.478	VV	0.2005	102.29539	6.68612	1.1851
21	11.644	VV	0.1468	74.70157	7.09774	0.8655
22	12.039	VV	0.4011	302.13675	9.29713	3.5004
23	12.670	VV	0.1155	261.99240	33.42233	3.0353
24	12.897	VV	0.1198	44.73871	5.22966	0.5183
25	13.097	VV	0.1515	57.32133	5.32748	0.6641
26	13.523	VV	0.2493	182.46095	9.12453	2.1139
27	13.830	VV	0.0924	34.07450	5.20153	0.3948
28	14.001	VV	0.2092	261.96762	18.50506	3.0350
29	14.341	VV	0.1087	42.18356	5.55068	0.4887
30	14.476	VV	0.1695	69.71514	5.58866	0.8077
31	14.768	VV	0.1808	85.24422	6.42613	0.9876
32	15.045	VB	0.1894	131.33948	9.04580	1.5216
33	15.426	BV	0.1875	45.72694	3.22371	0.5298
34	15.611	VV	0.1269	27.81105	3.15403	0.3222
35	15.752	VV	0.0881	16.81036	2.71903	0.1948
36	15.980	VV	0.1378	38.87465	3.72368	0.4504
37	16.091	VV	0.1046	41.89039	5.64491	0.4853
38	16.289	VV	0.1458	38.05185	4.04309	0.4408
39	16.574	VV	0.1261	227.62935	25.49887	2.6372
40	16.713	VV	0.0954	77.08742	11.93441	0.8931
41	16.871	VV	0.0952	57.00729	8.61623	0.6605
42	17.074	VV	0.1075	1537.43042	209.72937	17.8118
43	17.419	VV	0.1488	190.91740	18.43251	2.2119
44	17.665	VV	0.1249	96.24016	10.70382	1.1150
45	17.874	VV	0.1090	50.42392	6.46358	0.5842
46	18.057	VB	0.1907	151.08530	10.57329	1.7504
47	18.587	BV	0.1144	59.73447	7.22690	0.6921
48	18.697	VV	0.1271	71.64899	7.95585	0.8301
49	18.972	VB	0.1469	286.98947	27.70441	3.3249
50	19.542	BV	0.1454	48.84924	4.69503	0.5659
51	19.863	VV	0.1211	113.19756	13.59650	1.3114
52	20.096	VB	0.1247	595.52045	69.02480	6.8994
53	21.388	BV	0.1661	359.42303	32.24258	4.1641
54	21.950	VV	0.1655	77.19665	6.45418	0.8944
55	22.163	VV	0.1419	55.53376	5.59014	0.6434
56	22.386	VV	0.2052	103.30909	7.04084	1.1969
57	22.671	VB	0.1687	40.56932	3.36307	0.4700
58	23.188	BV	0.1832	18.22354	1.40615	0.2111
59	23.435	VV	0.1262	11.52437	1.34163	0.1335
60	23.676	VB	0.1471	50.61243	4.95586	0.5864
61	24.366	BV	0.3026	48.66038	2.19538	0.5638
62	24.889	VV	0.1821	80.63279	5.95085	0.9342
63	25.232	VB	0.2255	42.98893	2.48945	0.4980
64	25.770	BV	0.2241	38.34752	2.45670	0.4443
65	26.296	VV	0.2471	34.04451	1.79624	0.3944
66	26.950	VB	0.1722	165.20374	13.17887	1.9140
67	27.593	BB	0.2108	159.03944	10.03796	1.8425
68	28.243	BV	0.2025	99.19340	6.70995	1.1492
69	28.515	VB	0.2950	91.30144	3.95910	1.0578

Daidzein: 1



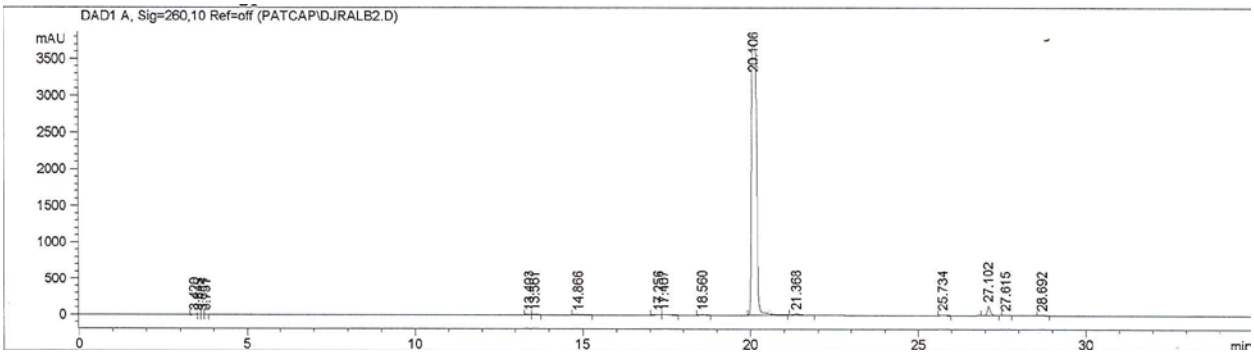
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	13.535	BV	0.1693	22.45639	1.99612	0.0559
2	13.701	VB	0.1221	10.24693	1.19371	0.0255
3	14.915	BV	0.1473	10.71107	1.06517	0.0267
4	15.117	VP	0.2305	22.94422	1.48261	0.0571
5	17.458	PV	0.2606	182.78271	11.18767	0.4550
6	17.632	VB	0.1719	116.63168	9.45751	0.2903
7	18.795	PP	0.1266	97.46799	12.02138	0.2426
8	20.061	PB	0.1683	3.81224e4	3696.48291	94.8952
9	21.531	BB	0.1592	218.37097	21.02262	0.5436
10	22.386	BV	0.4226	48.60470	1.50558	0.1210
11	25.881	PB	0.1392	35.14184	3.75472	0.0875
12	26.943	BV	0.1102	13.43039	1.81709	0.0334
13	27.134	VB	0.1111	862.59778	118.30792	2.1472
14	27.552	BB	0.2797	79.70473	3.69453	0.1984
15	28.235	BB	0.2658	161.69731	8.20792	0.4025
16	28.873	BP	0.1560	18.34926	1.72613	0.0457
17	33.294	BB	0.4214	149.62679	5.33213	0.3725

Daidzein: 2



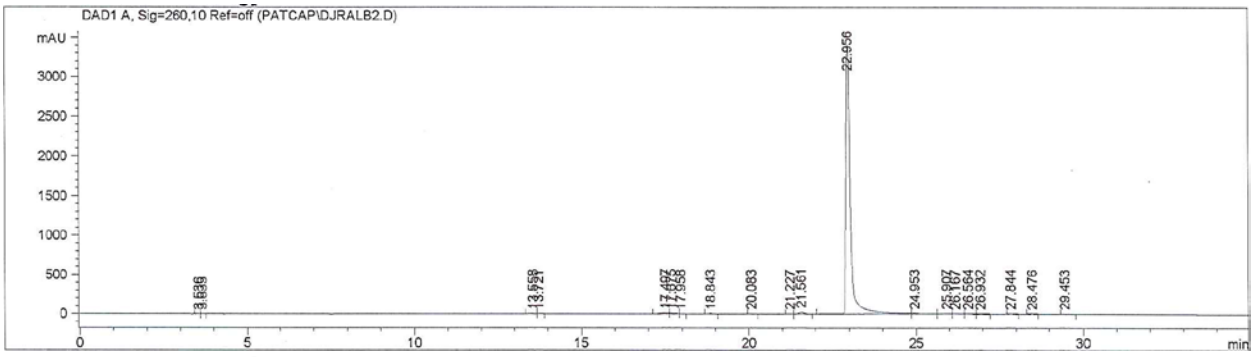
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.397	PV	0.0647	8.18223	2.46587	0.0197
2	3.509	VV	0.0709	10.89539	2.23250	0.0263
3	3.615	VV	0.1244	11.50961	1.48604	0.0278
4	3.743	VB	0.0717	5.34214	1.04456	0.0129
5	13.412	BV	0.1276	12.50037	1.49351	0.0301
6	13.575	VB	0.1161	9.07683	1.14926	0.0219
7	14.886	BP	0.2422	20.61807	1.12274	0.0497
8	17.254	BV	0.1869	104.70219	8.66764	0.2525
9	17.425	VB	0.1945	106.13850	7.62197	0.2560
10	18.583	BB	0.1292	98.41813	11.81574	0.2373
11	20.120	PB	0.1735	3.98663e4	3707.07861	96.1417
12	21.380	BB	0.1886	224.36005	17.61258	0.5411
13	22.981	VP	0.1568	17.81721	1.61410	0.0430
14	25.759	PP	0.1248	29.43040	3.62017	0.0710
15	27.125	BB	0.1088	918.27698	126.27887	2.2145
16	27.636	BB	0.1296	12.95789	1.45981	0.0312
17	28.719	BP	0.1366	9.65947	1.07763	0.0233

Daidzein: 3



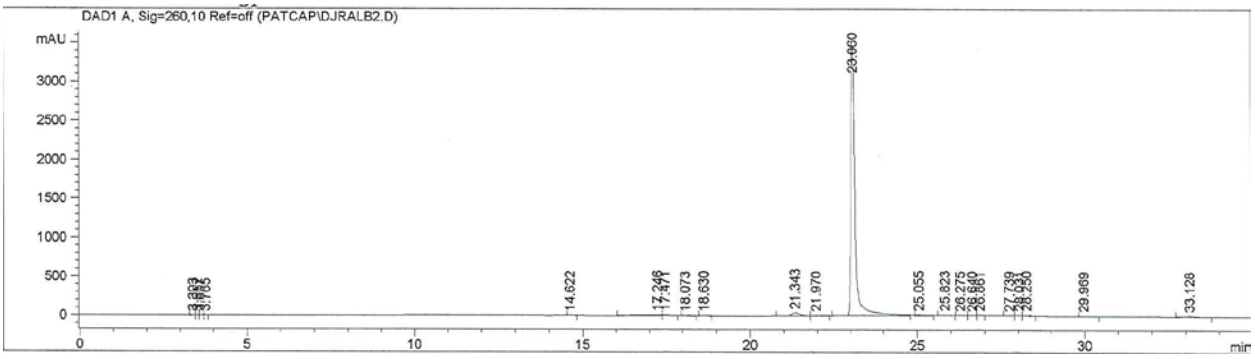
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.420	PV	0.0897	18.94642	2.92081	0.0459
2	3.545	VV	0.0732	10.15951	1.93818	0.0246
3	3.664	VV	0.0869	9.08143	1.49337	0.0220
4	3.751	VB	0.0720	5.44002	1.05706	0.0132
5	13.403	BV	0.1112	7.97923	1.11875	0.0193
6	13.561	VB	0.1161	8.21376	1.06388	0.0199
7	14.866	BP	0.2353	20.18401	1.14722	0.0489
8	17.256	BV	0.1518	77.00273	7.62426	0.1867
9	17.407	VB	0.1927	102.97842	7.47754	0.2497
10	18.560	BP	0.1277	99.72339	12.16139	0.2418
11	20.106	PB	0.1732	3.96401e4	3694.43066	96.1213
12	21.368	BB	0.2090	263.62784	17.78050	0.6393
13	25.734	PP	0.1228	29.57734	3.63986	0.0717
14	27.102	BB	0.1092	921.61200	129.24289	2.2348
15	27.615	BB	0.1385	16.09972	1.64047	0.0390
16	28.692	BP	0.1332	8.94454	1.03125	0.0217

Genistein: 1



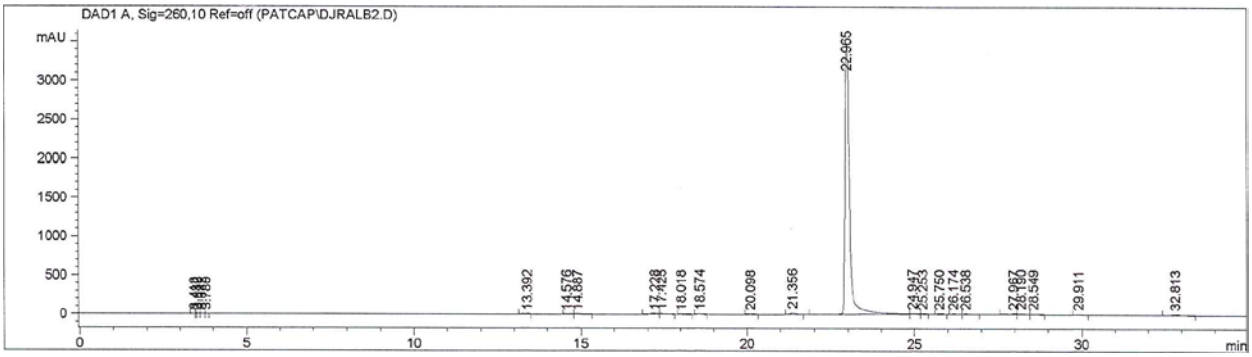
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.536	VV	0.0966	11.84401	1.71347	0.0355
2	3.635	VV	0.1047	10.64818	1.43308	0.0319
3	13.558	BV	0.1660	24.73194	2.28974	0.0741
4	13.721	VB	0.1196	10.78918	1.28907	0.0323
5	17.497	BV	0.2783	206.44467	12.02365	0.6185
6	17.675	VV	0.1629	110.38234	9.54782	0.3307
7	17.958	VB	0.1031	16.64058	2.33696	0.0499
8	18.843	BP	0.1276	36.15731	4.41268	0.1083
9	20.083	PB	0.1108	14.61838	1.96479	0.0438
10	21.227	PV	0.1181	14.82042	1.83744	0.0444
11	21.561	VB	0.1755	274.44565	23.62262	0.8222
12	22.956	BV	0.1415	3.20880e4	3418.51196	96.1313
13	24.953	VV	0.3697	294.38443	10.05191	0.8819
14	25.907	VV	0.3040	107.13972	4.59604	0.3210
15	26.167	VV	0.2067	49.87450	3.21999	0.1494
16	26.564	VV	0.1731	34.84895	2.80296	0.1044
17	26.932	VB	0.2186	19.00077	1.16312	0.0569
18	27.844	BB	0.1273	16.72756	1.96547	0.0501
19	28.476	BV	0.1433	22.21010	2.37168	0.0665
20	29.453	VP	0.1495	15.64503	1.55354	0.0469

Genistein: 2



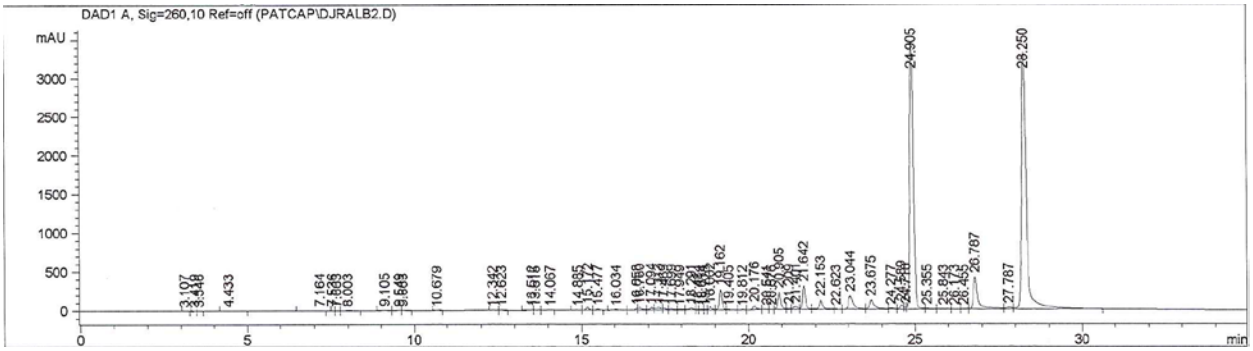
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.393	PV	0.0915	19.94005	3.78338	0.0574
2	3.517	VV	0.0868	15.26781	2.58940	0.0439
3	3.607	VV	0.1107	16.17355	1.87412	0.0465
4	3.765	VB	0.0689	5.69990	1.16791	0.0164
5	14.622	BB	0.1340	12.45167	1.25211	0.0358
6	17.246	PV	0.6718	563.61932	10.34264	1.6214
7	17.471	VB	0.2069	141.89938	9.68856	0.4082
8	18.073	BB	0.1629	21.96811	1.81877	0.0632
9	18.630	BB	0.1420	47.72348	5.06160	0.1373
10	21.343	BB	0.2592	605.81915	36.97264	1.7428
11	21.970	BB	0.3603	83.93430	3.07668	0.2415
12	23.060	BB	0.1413	3.23890e4	3458.26660	93.1753
13	25.055	BB	0.2886	221.07741	9.82224	0.6360
14	25.823	BB	0.2646	128.69731	6.80580	0.3702
15	26.275	BV	0.2014	45.87384	3.01667	0.1320
16	26.640	VV	0.1546	38.07608	3.45457	0.1095
17	26.861	VV	0.1420	23.28982	2.30258	0.0670
18	27.739	BV	0.1981	23.55744	1.65449	0.0678
19	28.031	VV	0.1460	52.11526	4.98310	0.1499
20	28.250	VV	0.1838	93.97540	7.22033	0.2703
21	29.969	VB	0.1863	20.58372	1.57651	0.0592
22	33.128	BB	0.3827	190.60214	7.54687	0.5483

Genistein: 3



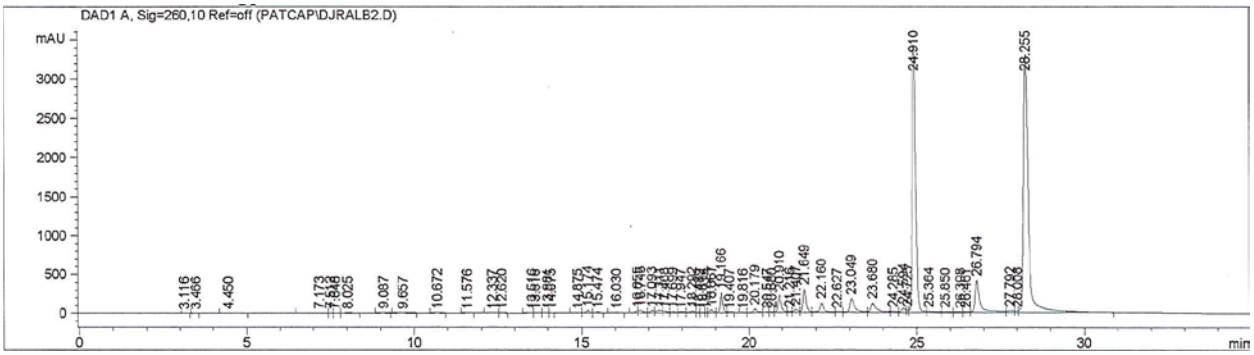
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.413	PV	0.0774	15.37763	2.74307	0.0450
2	3.482	VV	0.0502	8.11498	2.35332	0.0238
3	3.546	VV	0.0628	10.33227	2.37329	0.0303
4	3.642	VV	0.0940	12.96625	1.93833	0.0380
5	3.780	VB	0.0777	7.12038	1.26365	0.0209
6	13.392	BV	0.1759	17.58674	1.60404	0.0515
7	14.576	BB	0.1467	11.48121	1.04090	0.0336
8	14.887	BP	0.2373	24.47458	1.37721	0.0717
9	17.228	BV	0.2744	161.30908	9.48361	0.4724
10	17.425	VB	0.2018	114.09978	7.83963	0.3342
11	18.018	BB	0.1117	12.75949	1.65884	0.0374
12	18.574	BB	0.1300	37.36464	4.44660	0.1094
13	20.098	PB	0.1157	11.79668	1.50101	0.0345
14	21.356	BB	0.1825	241.05367	20.30552	0.7060
15	22.965	BV	0.1433	3.25221e4	3472.13306	95.2490
16	24.947	VV	0.2037	157.64891	10.23360	0.4617
17	25.253	VB	0.1752	78.77065	6.07882	0.2307
18	25.750	BB	0.2065	100.80607	6.59025	0.2952
19	26.174	BV	0.2286	77.42123	4.45726	0.2267
20	26.538	VV	0.2187	67.80267	4.10436	0.1986
21	27.967	BV	0.2055	80.89513	5.25740	0.2369
22	28.190	VV	0.1812	107.41640	8.28902	0.3146
23	28.549	VP	0.1576	34.15560	3.02885	0.1000
24	29.911	PB	0.1502	14.08290	1.41370	0.0412
25	32.813	BB	0.3467	217.36253	9.36629	0.6366

Promensil: 1



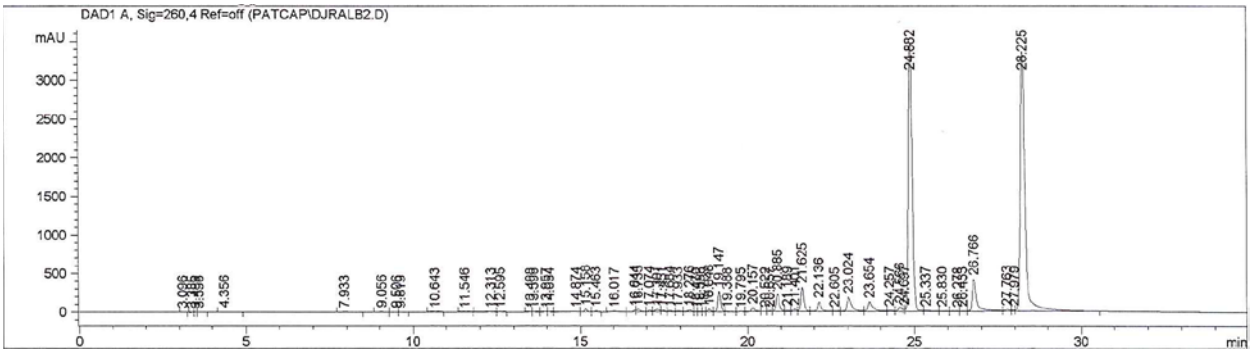
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.107	BB	0.1332	15.21019	1.48913	0.0195
2	3.419	PV	0.0811	9.49672	2.35314	0.0122
3	3.546	VV	0.1047	12.94689	1.78257	0.0166
4	4.433	BP	0.2834	35.77159	1.62143	0.0458
5	7.164	BV	0.4725	62.01506	1.66500	0.0794
6	7.536	VV	0.0728	8.54355	1.75588	0.0109
7	7.663	VP	0.0838	8.09360	1.43579	0.0104
8	8.003	VB	0.2208	86.12922	5.88249	0.1102
9	9.105	PB	0.1101	58.66859	7.27741	0.0751
10	9.549	BV	0.1504	25.88761	2.50886	0.0331
11	9.663	VB	0.1288	23.13085	2.43626	0.0296
12	10.679	BB	0.1022	22.18979	3.31132	0.0284
13	12.342	BV	0.1285	14.57553	1.59606	0.0187
14	12.623	VP	0.0887	39.03024	6.82747	0.0499
15	13.512	PV	0.1490	45.59528	4.12207	0.0583
16	13.615	VV	0.1076	25.23669	3.35929	0.0323
17	14.067	VV	0.2046	63.37617	3.96206	0.0811
18	14.885	BV	0.2071	75.98106	5.30447	0.0972
19	15.172	VV	0.0947	239.22264	37.35822	0.3061
20	15.477	VP	0.0904	91.45496	15.14639	0.1170
21	16.034	VV	0.1538	78.17511	7.36986	0.1000
22	16.658	VV	0.0785	119.39252	23.03363	0.1528
23	16.750	VV	0.1213	287.46149	33.10460	0.3679
24	17.094	VV	0.1104	244.15100	31.51078	0.3124
25	17.313	VV	0.1638	372.73386	31.55308	0.4770
26	17.469	VV	0.1164	169.39491	20.94436	0.2168
27	17.699	VV	0.1247	161.36046	17.97347	0.2065
28	17.949	VV	0.1310	106.95325	10.85041	0.1369
29	18.291	VV	0.1168	194.14621	23.40397	0.2484
30	18.484	VV	0.0866	26.94778	4.58315	0.0345
31	18.614	VV	0.1094	48.10726	6.42445	0.0616
32	18.725	VV	0.0768	30.86992	5.92583	0.0395
33	18.862	VV	0.0915	230.12444	37.57440	0.2945
34	19.162	VV	0.0881	1544.65198	264.79373	1.9767
35	19.405	VV	0.1469	48.82518	4.48841	0.0625
36	19.812	VV	0.1410	28.07663	2.75298	0.0359
37	20.176	VV	0.1086	364.59140	49.13213	0.4666
38	20.541	VV	0.1357	62.47601	6.19278	0.0800
39	20.676	VV	0.1114	51.69294	6.74800	0.0662
40	20.905	VV	0.1079	1657.81836	230.62932	2.1215
41	21.209	VV	0.1021	108.03355	15.34402	0.1382
42	21.401	VV	0.1548	406.73798	39.95604	0.5205
43	21.642	VV	0.0977	2007.63293	309.49063	2.5692
44	22.153	VV	0.1320	1149.26172	124.17799	1.4707
45	22.623	VV	0.1746	82.67210	6.49053	0.1058
46	23.044	VV	0.1466	1862.41821	177.29262	2.3833
47	23.675	VV	0.1612	1421.94885	122.67740	1.8197
48	24.277	VV	0.1677	156.10777	13.03401	0.1998
49	24.589	VV	0.1080	419.98169	58.34601	0.5374
50	24.718	VV	0.0607	116.19755	27.82262	0.1487
51	24.905	VV	0.1168	2.57640e4	3461.02490	32.9700
52	25.355	VV	0.1834	170.54439	12.32385	0.2182
53	25.843	VV	0.2826	141.24959	6.32438	0.1808
54	26.173	VV	0.2122	63.83978	3.91563	0.0817
55	26.455	VV	0.1627	55.39117	4.52989	0.0709
56	26.787	VB	0.1426	4118.94922	412.00446	5.2710
57	27.787	BV	0.1703	296.32620	24.30304	0.3792
58	28.250	VB	0.1498	3.30119e4	3327.23999	42.2452

Promensil: 2



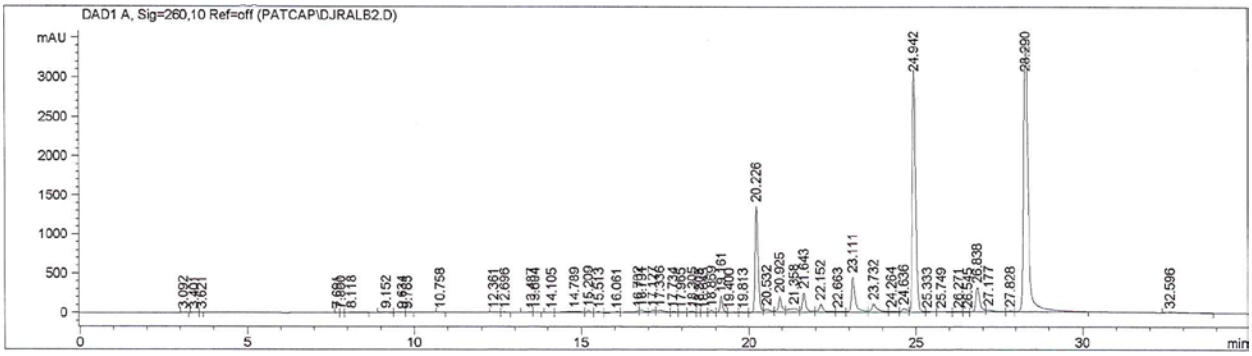
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.116	BB	0.1208	12.12884	1.32473	0.0152
2	3.466	PB	0.0907	10.76779	1.55719	0.0135
3	4.450	BP	0.2904	36.23555	1.61100	0.0453
4	7.173	BV	0.4894	65.70956	1.69830	0.0821
5	7.512	VV	0.0836	13.59213	2.41800	0.0170
6	7.648	VP	0.0910	10.33223	1.65065	0.0129
7	8.025	VB	0.2276	85.06333	5.65178	0.1063
8	9.087	BB	0.1126	57.27628	7.06339	0.0715
9	9.657	BB	0.2549	53.28251	2.62401	0.0666
10	10.672	BB	0.1194	30.21365	3.61785	0.0377
11	11.576	PB	0.1500	11.90612	1.06810	0.0149
12	12.337	BV	0.1957	36.15962	2.42876	0.0452
13	12.620	VP	0.0955	42.19503	6.88516	0.0527
14	13.516	PV	0.1429	35.92608	3.46583	0.0449
15	13.616	VB	0.0986	18.67467	2.77280	0.0233
16	13.981	BV	0.1124	17.08368	2.25536	0.0213
17	14.075	VP	0.0816	12.80638	2.34745	0.0160
18	14.875	BV	0.1874	46.55013	3.95146	0.0581
19	15.174	VV	0.0898	210.70256	35.22170	0.2632
20	15.474	VP	0.0890	84.11533	14.22932	0.1051
21	16.030	VB	0.1548	75.83863	7.20622	0.0947
22	16.655	BV	0.0768	120.42236	23.91296	0.1504
23	16.745	VV	0.1211	293.89801	33.90493	0.3671
24	17.093	VV	0.1097	239.99590	31.20374	0.2998
25	17.311	VV	0.1661	379.81812	31.62052	0.4744
26	17.468	VV	0.1171	174.77977	21.44555	0.2183
27	17.699	VV	0.1297	168.15633	18.22221	0.2100
28	17.947	VV	0.1349	114.77414	11.45264	0.1434
29	18.292	VV	0.1204	205.12424	23.83689	0.2562
30	18.489	VV	0.0840	29.45293	5.05126	0.0368
31	18.614	VV	0.1093	55.28167	7.22557	0.0691
32	18.724	VV	0.0785	35.23435	6.58086	0.0440
33	18.867	VV	0.0960	239.80342	37.80758	0.2995
34	19.166	VV	0.0915	1567.67419	263.21991	1.9582
35	19.407	VB	0.1247	48.84789	5.44185	0.0610
36	19.816	BV	0.1507	40.90158	3.70642	0.0511
37	20.179	VV	0.1156	397.68027	49.59277	0.4968
38	20.547	VV	0.1399	78.95432	7.55822	0.0986
39	20.680	VV	0.1128	65.07249	8.18248	0.0813
40	20.910	VV	0.1103	1711.30542	231.21799	2.1376
41	21.216	VV	0.1011	120.74605	16.95885	0.1508
42	21.407	VV	0.1583	434.55820	41.46445	0.5428
43	21.649	VV	0.1002	2066.36963	308.14816	2.5812
44	22.160	VV	0.1394	1236.88757	125.04625	1.5450
45	22.627	VV	0.1806	117.86852	8.89205	0.1472
46	23.049	VV	0.1535	1998.72449	180.10252	2.4967
47	23.680	VV	0.1700	1553.52783	125.82590	1.9406
48	24.285	VV	0.1784	202.71541	15.94037	0.2532
49	24.594	VV	0.1098	457.31769	60.75808	0.5713
50	24.725	VV	0.0635	133.26393	31.39709	0.1665
51	24.910	VV	0.1187	2.61450e4	3438.21606	32.6586
52	25.364	VB	0.2322	281.15735	15.46082	0.3512
53	25.850	BV	0.2253	162.97231	9.35445	0.2036
54	26.308	VV	0.2147	118.22410	7.01228	0.1477
55	26.461	VV	0.1684	100.82878	7.82294	0.1259
56	26.794	VV	0.1480	4368.55176	417.82181	5.4569
57	27.792	VV	0.1701	321.68738	26.03936	0.4018
58	28.008	VV	0.0847	113.15290	19.21030	0.1413
59	28.255	VB	0.1512	3.31882e4	3303.32666	41.4565

Promensil: 3



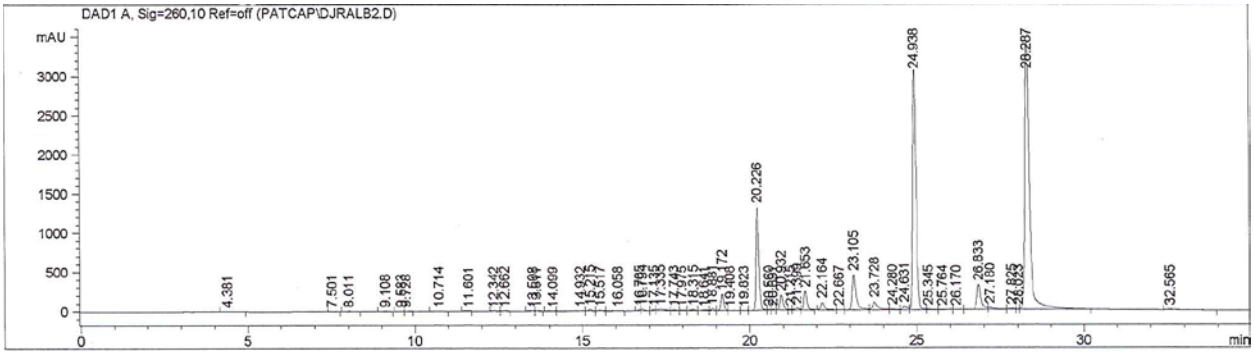
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.096	BB	0.1316	14.95706	1.50944	0.0188
2	3.385	PV	0.0620	7.08200	2.17495	8.897e-3
3	3.489	VV	0.0724	7.27697	1.50436	9.142e-3
4	3.596	VB	0.1267	11.98905	1.21951	0.0151
5	4.356	BP	0.2700	37.83652	1.79454	0.0475
6	7.933	VB	0.2294	96.79562	6.22686	0.1216
7	9.056	BB	0.1102	58.14470	7.19982	0.0730
8	9.506	BV	0.1442	25.08412	2.51889	0.0315
9	9.619	VB	0.1283	22.20467	2.39143	0.0279
10	10.643	BP	0.1097	23.79043	3.31422	0.0299
11	11.546	PB	0.1758	12.03754	1.01923	0.0151
12	12.313	BV	0.2974	72.86727	3.10947	0.0915
13	12.595	VB	0.0994	50.45551	7.60146	0.0634
14	13.498	BV	0.1023	22.61600	3.36976	0.0284
15	13.596	VV	0.1050	22.50797	3.09004	0.0283
16	13.957	VV	0.1188	20.12224	2.52890	0.0253
17	14.054	VP	0.0837	14.82250	2.63254	0.0186
18	14.874	VV	0.2872	109.48799	5.04551	0.1375
19	15.158	VV	0.0952	234.59032	36.40063	0.2947
20	15.463	VV	0.0893	91.46853	14.96245	0.1149
21	16.017	VV	0.1571	77.83339	7.14615	0.0978
22	16.644	VV	0.0748	119.17879	23.65820	0.1497
23	16.735	VV	0.1206	291.77875	33.84421	0.3666
24	17.074	VV	0.0921	135.68544	21.94606	0.1705
25	17.301	VV	0.1512	306.38895	28.55276	0.3849
26	17.451	VV	0.1150	156.26810	19.59620	0.1963
27	17.684	VV	0.1245	160.30367	17.88975	0.2014
28	17.933	VV	0.1316	112.16262	11.32073	0.1409
29	18.276	VV	0.1200	200.66360	23.41229	0.2521
30	18.470	VV	0.0861	28.08234	4.81359	0.0353
31	18.596	VV	0.1076	48.45877	6.60472	0.0609
32	18.709	VV	0.0798	34.29414	6.27589	0.0431
33	18.848	VV	0.0945	235.47795	37.86828	0.2958
34	19.147	VV	0.0888	1571.54028	266.67160	1.9743
35	19.388	VV	0.1541	59.72304	5.19524	0.0750
36	19.795	VV	0.1546	39.26505	3.40297	0.0493
37	20.157	VV	0.1124	388.57968	50.16235	0.4882
38	20.522	VV	0.1405	73.25865	7.09494	0.0920
39	20.657	VV	0.1115	60.71256	7.74402	0.0763
40	20.885	VV	0.1090	1693.51953	232.31824	2.1275
41	21.189	VV	0.1183	130.25772	16.11617	0.1636
42	21.401	VV	0.1440	286.03741	29.80816	0.3593
43	21.625	VV	0.0989	2091.40698	317.37036	2.6274
44	22.136	VV	0.1347	1190.29370	127.72018	1.4953
45	22.605	VV	0.1810	103.70614	7.80797	0.1303
46	23.024	VV	0.1486	1993.46814	186.63907	2.5043
47	23.654	VV	0.1626	1526.78723	128.55060	1.9180
48	24.257	VV	0.1738	178.58873	14.49097	0.2244
49	24.566	VV	0.1100	441.65170	59.91039	0.5548
50	24.697	VV	0.0616	129.24098	30.40309	0.1624
51	24.882	VV	0.1177	2.60765e4	3470.76733	32.7590
52	25.337	VV	0.2365	253.49982	13.78490	0.3185
53	25.830	VV	0.2156	129.00201	7.69574	0.1621
54	26.278	VV	0.2183	97.88474	5.75930	0.1230
55	26.433	VV	0.1689	84.61040	6.63042	0.1063
56	26.766	VV	0.1440	4244.76904	419.58179	5.3326
57	27.763	VV	0.1660	292.06921	24.34163	0.3669
58	27.979	VV	0.0846	104.02189	17.69266	0.1307
59	28.225	VB	0.1503	3.34979e4	3360.60010	42.0823

Promensil, spiked: 1



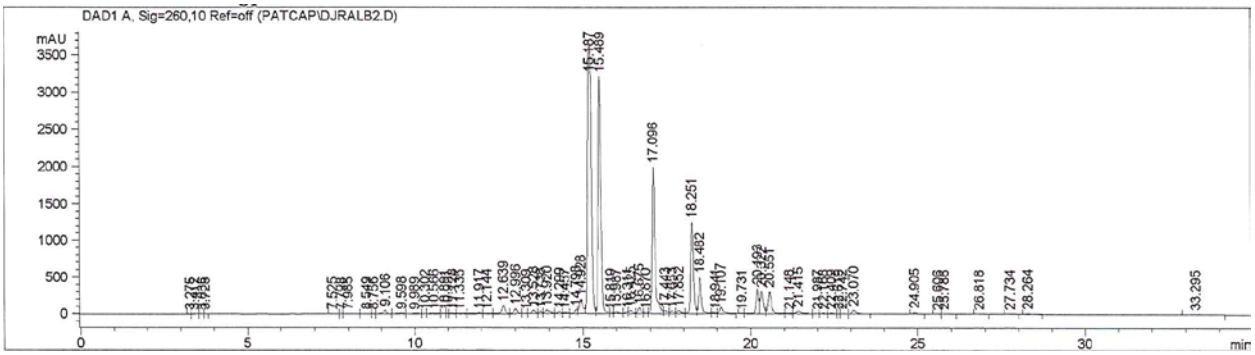
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.092	BB	0.1420	14.55126	1.32643	0.0174
2	3.401	PV	0.1335	24.18710	2.77907	0.0289
3	3.621	VV	0.0820	7.54859	1.29446	9.010e-3
4	7.691	PV	0.0506	7.89872	2.51969	9.428e-3
5	7.800	VV	0.0923	10.99314	1.68188	0.0131
6	8.118	VB	0.2206	87.34065	5.76858	0.1042
7	9.152	BB	0.1106	44.03723	5.42839	0.0526
8	9.634	BV	0.1856	22.77557	1.80035	0.0272
9	9.785	VP	0.1085	9.69693	1.27766	0.0116
10	10.758	PB	0.1046	17.04401	2.46619	0.0203
11	12.361	BP	0.1303	11.36676	1.40648	0.0136
12	12.696	VP	0.0919	32.08131	5.50886	0.0383
13	13.487	PV	0.1818	33.64648	2.84847	0.0402
14	13.604	VB	0.1037	18.87653	2.76385	0.0225
15	14.105	BV	0.1386	38.55650	3.79242	0.0460
16	14.789	VV	0.4509	482.29367	13.43256	0.5757
17	15.209	VV	0.0909	176.14859	28.98034	0.2102
18	15.513	VP	0.0921	70.32097	11.38078	0.0839
19	16.061	VV	0.1415	64.10651	6.70632	0.0765
20	16.702	VV	0.1528	289.36255	25.04490	0.3454
21	16.791	VV	0.1333	307.36469	31.65969	0.3669
22	17.127	VV	0.1265	280.82098	30.75990	0.3352
23	17.336	VV	0.2111	455.66675	27.82515	0.5439
24	17.734	VV	0.1199	114.01004	13.31935	0.1361
25	17.965	VV	0.1357	77.11614	7.77517	0.0920
26	18.305	VV	0.1151	146.13211	18.30544	0.1744
27	18.505	VV	0.0823	20.92097	3.67935	0.0250
28	18.645	VV	0.1585	67.50578	5.94513	0.0806
29	18.869	VV	0.0899	173.96143	29.03854	0.2076
30	19.161	VV	0.0894	1202.13293	208.16718	1.4348
31	19.400	VV	0.1540	38.96410	3.34219	0.0465
32	19.813	VV	0.1528	24.44490	2.21504	0.0292
33	20.226	VV	0.0937	8355.54297	1358.96704	9.9729
34	20.532	VV	0.2179	577.69861	37.45507	0.6895
35	20.925	VV	0.1132	1595.89258	204.24072	1.9048
36	21.358	VV	0.2507	960.87762	52.20238	1.1469
37	21.643	VV	0.1097	1915.07043	254.84840	2.2858
38	22.152	VV	0.1430	1086.46338	106.47433	1.2968
39	22.663	VV	0.2356	162.53944	9.59822	0.1940
40	23.111	VV	0.1253	3917.97900	451.01227	4.6764
41	23.732	VV	0.1644	1289.47070	107.14465	1.5391
42	24.264	VV	0.1923	198.88828	13.95379	0.2374
43	24.636	VV	0.1318	445.39282	47.32322	0.5316
44	24.942	VV	0.1091	2.14991e4	3093.29150	25.6607
45	25.333	VV	0.1712	124.12613	9.70308	0.1482
46	25.749	VV	0.2459	80.38997	4.55581	0.0960
47	26.271	VV	0.1997	17.50712	1.16226	0.0209
48	26.545	VV	0.1244	12.68160	1.60086	0.0151
49	26.838	VV	0.1213	2631.18237	315.63913	3.1405
50	27.177	VV	0.2221	475.08228	27.97811	0.5670
51	27.828	VV	0.1539	163.04939	15.10989	0.1946
52	28.290	VB	0.1495	3.37109e4	3404.87939	40.2364
53	32.596	BP	0.3227	190.43365	7.97268	0.2273

Promensil, spiked: 2



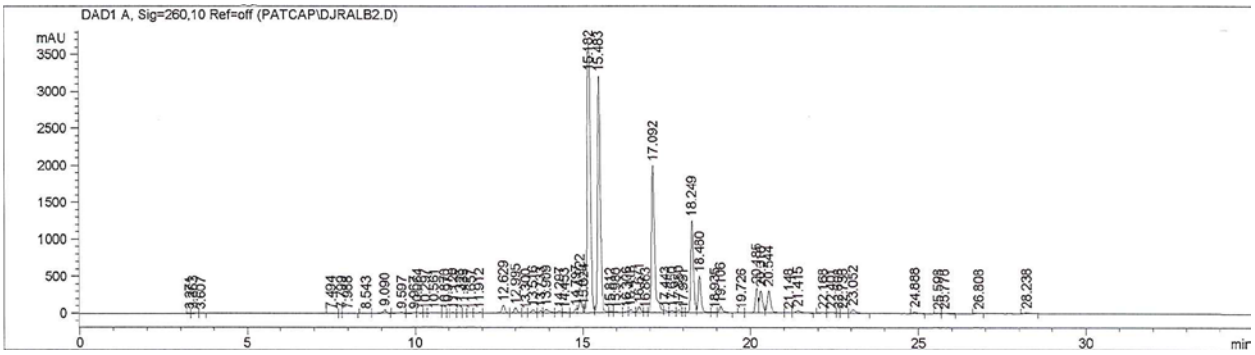
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.381	BP	0.2815	31.89273	1.45619	0.0393
2	7.501	PP	0.1217	18.57153	2.17372	0.0229
3	8.011	PB	0.2185	69.19765	4.73565	0.0853
4	9.108	BB	0.1140	44.29646	5.27476	0.0546
5	9.583	BV	0.1440	17.04916	1.77561	0.0210
6	9.728	VB	0.1151	11.49270	1.38017	0.0142
7	10.714	PB	0.1204	20.27864	2.45492	0.0250
8	11.601	BV	0.1512	10.20790	1.03417	0.0126
9	12.342	BV	0.1851	36.81514	2.88015	0.0454
10	12.662	VB	0.1006	37.66197	5.59236	0.0464
11	13.508	BV	0.1241	21.97399	2.72540	0.0271
12	13.611	VB	0.1100	20.98957	2.78148	0.0259
13	14.099	BV	0.1565	21.79259	1.86229	0.0269
14	14.932	VV	0.3048	114.00523	4.87526	0.1405
15	15.215	VV	0.0946	170.27249	27.37191	0.2098
16	15.517	VP	0.0974	71.62874	11.08846	0.0883
17	16.058	VV	0.1509	55.96370	5.58403	0.0690
18	16.705	BV	0.0736	92.68378	19.45888	0.1142
19	16.794	VV	0.1175	220.19652	26.35431	0.2713
20	17.135	VV	0.0975	133.34773	20.06687	0.1643
21	17.335	VV	0.2192	424.96027	24.88615	0.5236
22	17.743	VV	0.1251	118.76706	13.43375	0.1463
23	17.975	VV	0.1330	81.31571	8.25227	0.1002
24	18.315	VV	0.1167	147.75781	18.19418	0.1821
25	18.641	VV	0.1943	87.39761	5.98584	0.1077
26	18.881	VV	0.0928	175.59468	28.94309	0.2163
27	19.172	VV	0.0907	1213.78601	206.22263	1.4955
28	19.408	VB	0.1290	29.67384	3.17714	0.0366
29	19.823	BV	0.1183	12.17225	1.50552	0.0150
30	20.226	VV	0.0958	8122.50635	1318.90942	10.0076
31	20.560	VV	0.0905	52.26101	8.18073	0.0644
32	20.691	VV	0.1150	64.23051	7.89114	0.0791
33	20.932	VV	0.1031	1328.85425	191.21706	1.6373
34	21.215	VV	0.0981	85.86880	12.50331	0.1058
35	21.399	VV	0.1574	318.94138	30.64200	0.3930
36	21.653	VB	0.0998	1615.87122	242.31406	1.9909
37	22.164	BV	0.1264	849.09381	98.64030	1.0462
38	22.667	VV	0.1640	60.48807	4.96990	0.0745
39	23.105	VV	0.1263	3859.73804	449.17841	4.7555
40	23.728	VV	0.1598	1235.50342	106.14156	1.5222
41	24.280	VV	0.1901	162.75635	11.85972	0.2005
42	24.631	VV	0.1342	452.54871	47.06530	0.5576
43	24.938	VV	0.1080	2.15894e4	3072.10864	26.6000
44	25.345	VV	0.1906	149.64960	10.35235	0.1844
45	25.764	VV	0.2843	112.05563	5.10074	0.1381
46	26.170	VV	0.2164	51.98772	3.08860	0.0641
47	26.833	VV	0.1267	2772.75220	321.29565	3.4163
48	27.180	VV	0.2273	492.30716	28.24850	0.6066
49	27.825	VV	0.1594	187.02760	16.35503	0.2304
50	28.023	VV	0.0870	63.90716	10.80978	0.0787
51	28.287	VB	0.1494	3.38123e4	3419.79248	41.6596
52	32.565	BP	0.3503	211.50014	8.00249	0.2606

Soy: 1



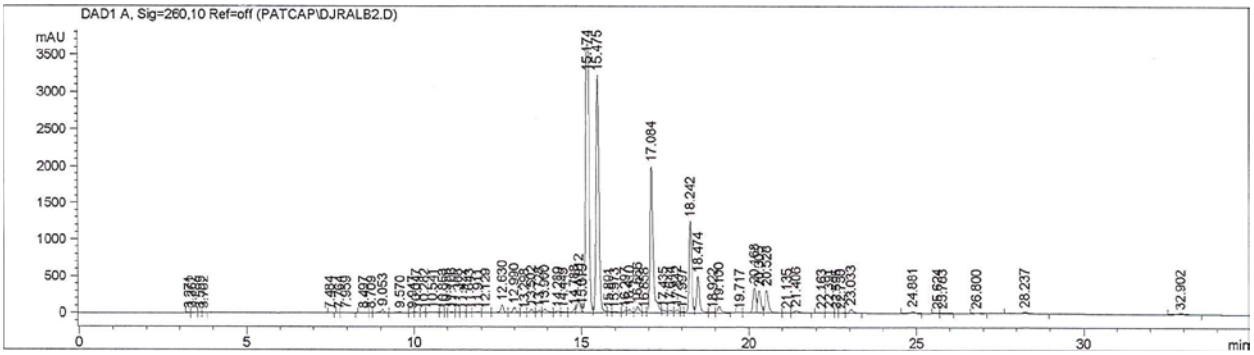
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.275	BP	0.0876	10.60109	1.94323	0.0123
2	3.412	VV	0.1200	44.01020	4.93697	0.0511
3	3.636	VV	0.1123	17.04437	2.02299	0.0198
4	3.728	VB	0.0735	6.29178	1.15595	7.302e-3
5	7.525	BV	0.1104	26.42196	3.48703	0.0307
6	7.798	VV	0.0694	9.71503	2.04662	0.0113
7	7.985	VB	0.2440	58.60932	3.28691	0.0680
8	8.549	BV	0.2274	40.90588	2.44209	0.0475
9	8.756	VV	0.0892	12.98948	2.06940	0.0151
10	9.106	VB	0.1032	343.98245	47.11443	0.3992
11	9.598	BV	0.1732	155.21765	11.66930	0.1801
12	9.989	VV	0.2760	108.92758	5.00272	0.1264
13	10.302	VV	0.1235	32.15819	3.55754	0.0373
14	10.566	VV	0.2184	132.24298	8.55301	0.1535
15	10.891	VV	0.1195	45.79283	5.16338	0.0531
16	10.991	VV	0.0576	20.05062	4.91455	0.0233
17	11.128	VV	0.1308	107.07005	12.15043	0.1243
18	11.335	VV	0.2077	83.55557	5.24806	0.0970
19	11.917	VV	0.1855	168.44884	12.01775	0.1955
20	12.144	VV	0.2271	114.83200	6.86717	0.1333
21	12.639	VV	0.1024	820.71741	116.22794	0.9524
22	12.996	VV	0.1071	525.99341	72.12846	0.6104
23	13.309	VV	0.1374	87.37397	8.68263	0.1014
24	13.528	VV	0.1117	362.88217	47.19566	0.4211
25	13.738	VV	0.1012	220.22417	32.42555	0.2556
26	13.920	VV	0.1449	529.77661	50.29980	0.6148
27	14.299	VV	0.1194	258.16235	30.31181	0.2996
28	14.457	VV	0.1308	172.32709	17.82270	0.2000
29	14.798	VV	0.0977	375.56323	56.39112	0.4358
30	14.928	VV	0.1006	1383.72461	200.22855	1.6058
31	15.187	VV	0.1224	2.75767e4	3637.79102	32.0026
32	15.489	VV	0.0880	1.81918e4	3216.53784	21.1114
33	15.819	VV	0.1070	151.50648	19.43007	0.1758
34	15.987	VV	0.1677	244.45708	19.84464	0.2837
35	16.311	VV	0.0894	185.51132	29.47071	0.2153
36	16.425	VV	0.1112	353.81537	45.24853	0.4106
37	16.675	VV	0.1046	599.80255	84.68940	0.6961
38	16.870	VV	0.1039	160.25398	21.77353	0.1860
39	17.096	VV	0.0950	1.24641e4	1991.41553	14.4644
40	17.443	VV	0.1202	305.17593	36.26611	0.3542
41	17.653	VV	0.1174	292.64722	35.04747	0.3396
42	17.852	VV	0.1264	403.36206	44.22497	0.4681
43	18.251	VV	0.0845	6932.76953	1254.22485	8.0454
44	18.482	VV	0.0900	3022.02002	503.34811	3.5070
45	18.941	VV	0.1519	155.06180	15.08595	0.1799
46	19.107	VB	0.1267	830.79181	90.80093	0.9641
47	19.731	BV	0.1431	84.24057	8.11838	0.0978
48	20.193	VV	0.0928	2121.10327	349.61496	2.4615
49	20.322	VV	0.0931	1931.46863	308.24246	2.2415
50	20.551	VV	0.1089	2265.66650	304.33124	2.6293
51	21.148	VV	0.1732	89.65216	6.74115	0.1040
52	21.415	VV	0.1806	450.17535	35.34963	0.5224
53	21.987	VV	0.1587	51.37723	4.32082	0.0596
54	22.168	VV	0.1855	61.31074	4.84998	0.0712
55	22.409	VV	0.1582	76.42612	6.54712	0.0887
56	22.619	VV	0.0828	15.55548	2.71874	0.0181
57	22.742	VV	0.1245	31.40047	3.50396	0.0364
58	23.070	VB	0.1327	426.05130	45.71070	0.4944
59	24.905	BP	0.1079	149.30658	21.25590	0.1733
60	25.606	VV	0.0992	7.36867	1.11341	8.551e-3
61	25.786	VB	0.1619	15.02517	1.28949	0.0174
62	26.818	PB	0.1327	10.46439	1.18873	0.0121
63	27.734	BB	0.1543	20.20567	1.92703	0.0234
64	28.264	BB	0.1495	103.26867	9.91818	0.1198
65	33.295	BB	0.5431	114.94864	2.99980	0.1334

Soy: 2



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.271	BP	0.0906	15.07368	2.64187	0.0181
2	3.363	VV	0.1082	51.22281	6.20995	0.0617
3	3.607	VB	0.1431	25.33407	2.44114	0.0305
4	7.494	BP	0.1139	19.31977	2.35069	0.0233
5	7.799	VV	0.0657	6.54492	1.53609	7.878e-3
6	7.988	VB	0.2174	40.57134	2.63852	0.0488
7	8.543	BV	0.1968	21.39013	1.51468	0.0257
8	9.090	VB	0.1003	319.00235	45.21262	0.3840
9	9.597	BV	0.1531	115.68430	9.99245	0.1392
10	9.967	VV	0.1645	39.66458	3.24788	0.0477
11	10.064	VB	0.1191	23.37547	2.69737	0.0281
12	10.297	BV	0.0969	10.51900	1.55542	0.0127
13	10.561	VV	0.1928	88.68602	6.68241	0.1067
14	10.870	VV	0.0915	19.21720	2.96899	0.0231
15	10.972	VV	0.0627	10.67150	2.45633	0.0128
16	11.120	VV	0.1163	72.94620	9.63928	0.0878
17	11.326	VV	0.1093	19.69068	2.63062	0.0237
18	11.435	VV	0.1039	16.63342	2.25787	0.0200
19	11.657	VV	0.1272	19.65778	2.17941	0.0237
20	11.912	VV	0.1180	71.59937	8.70550	0.0862
21	12.629	VV	0.1050	799.22498	109.64350	0.9620
22	12.995	VV	0.0951	458.46790	71.26332	0.5518
23	13.300	VV	0.1361	51.38539	5.16437	0.0618
24	13.516	VV	0.1053	302.00641	43.33027	0.3635
25	13.733	VV	0.0947	186.11458	29.87181	0.2240
26	13.909	VV	0.1347	455.78180	47.17494	0.5486
27	14.297	VV	0.1084	201.78325	26.64087	0.2429
28	14.453	VV	0.1205	119.84111	13.63453	0.1442
29	14.797	VV	0.0867	309.74164	52.61094	0.3728
30	14.922	VV	0.0866	1157.39697	196.82985	1.3931
31	15.024	VV	0.0403	179.90407	65.02972	0.2165
32	15.182	VV	0.1226	2.76611e4	3641.13306	33.2937
33	15.483	VV	0.0881	1.81883e4	3212.39575	21.8919
34	15.812	VV	0.1033	119.12488	15.55724	0.1434
35	15.980	VV	0.1529	165.39195	15.21013	0.1991
36	16.306	VV	0.0852	144.74771	25.13148	0.1742
37	16.418	VV	0.1031	297.34335	40.74418	0.3579
38	16.671	VV	0.0960	532.83899	81.80780	0.6413
39	16.863	VV	0.1016	122.30975	17.06309	0.1472
40	17.092	VV	0.0944	1.23540e4	1990.74683	14.8696
41	17.443	VV	0.1186	251.83083	30.42726	0.3031
42	17.650	VV	0.1106	224.33615	28.88056	0.2700
43	17.850	VV	0.0980	256.29575	38.33535	0.3085
44	17.991	VV	0.0784	47.37080	8.85908	0.0570
45	18.249	VV	0.0837	6833.69482	1251.92859	8.2252
46	18.480	VV	0.0874	2860.53564	494.87473	3.4430
47	18.935	VV	0.1451	79.73989	8.22384	0.0960
48	19.106	VB	0.1026	593.35590	83.75784	0.7142
49	19.726	PP	0.0666	5.13111	1.34091	6.176e-3
50	20.185	VV	0.0870	1968.18738	342.69760	2.3690
51	20.318	VV	0.0920	1867.78271	302.78244	2.2481
52	20.544	VV	0.1058	2123.41016	302.72296	2.5558
53	21.148	VV	0.1553	39.46443	3.40250	0.0475
54	21.415	VB	0.1609	338.80099	30.18554	0.4078
55	22.168	BV	0.1955	36.56638	2.70783	0.0440
56	22.401	VV	0.1306	45.96544	4.84831	0.0553
57	22.608	VV	0.0803	7.31614	1.32752	8.806e-3
58	22.736	VP	0.0938	13.33177	2.16534	0.0160
59	23.052	VB	0.1238	391.90921	45.79532	0.4717
60	24.888	BB	0.1045	145.09479	21.01847	0.1746
61	25.598	VV	0.0936	6.38120	1.04022	7.681e-3
62	25.776	VP	0.1598	14.05691	1.28293	0.0169
63	26.808	PV	0.1470	14.44667	1.49137	0.0174
64	28.238	BB	0.1441	103.45914	10.39781	0.1245

Soy: 3



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.271	BP	0.0906	13.97687	2.44920	0.0164
2	3.362	VV	0.1105	49.41890	5.85082	0.0581
3	3.599	VV	0.0880	16.00933	2.59348	0.0188
4	3.702	VB	0.0849	12.47234	1.99615	0.0147
5	7.484	VB	0.0855	8.76243	1.47103	0.0103
6	7.764	BV	0.0656	6.30393	1.48301	7.412e-3
7	7.959	VB	0.2049	36.92125	2.55020	0.0434
8	8.497	BV	0.1875	20.75368	1.42867	0.0244
9	8.709	VV	0.0798	6.29534	1.11583	7.402e-3
10	9.053	VB	0.1012	313.86768	44.03576	0.3690
11	9.570	BV	0.1787	139.18159	10.10748	0.1636
12	9.947	VV	0.1194	28.39648	3.47257	0.0334
13	10.047	VV	0.1121	26.30819	3.26096	0.0309
14	10.282	VV	0.1202	18.67040	2.09090	0.0220
15	10.541	VV	0.1811	100.33763	7.26680	0.1180
16	10.853	VV	0.1100	28.42777	3.60654	0.0334
17	10.955	VV	0.0682	14.87103	3.20116	0.0175
18	11.106	VV	0.1211	85.03047	10.65175	0.1000
19	11.308	VV	0.1161	26.91426	3.40896	0.0316
20	11.422	VB	0.1195	27.67609	3.18139	0.0325
21	11.643	BV	0.1266	27.12082	2.96920	0.0319
22	11.911	VV	0.1257	87.65656	9.67499	0.1031
23	12.129	VV	0.2185	83.18952	5.04198	0.0978
24	12.630	VV	0.1018	780.73077	111.31770	0.9180
25	12.990	VV	0.1001	494.11386	71.93252	0.5810
26	13.298	VV	0.1428	70.25544	6.67684	0.0826
27	13.502	VV	0.1062	327.76846	45.40731	0.3854
28	13.728	VV	0.0965	202.64671	30.91440	0.2383
29	13.900	VV	0.1373	496.32806	50.21119	0.5836
30	14.289	VV	0.1143	223.04665	27.59171	0.2623
31	14.449	VV	0.1269	145.26495	15.56491	0.1708
32	14.788	VV	0.0908	336.70392	53.98589	0.3959
33	14.912	VV	0.0870	1172.88843	198.35443	1.3791
34	15.019	VV	0.0429	200.95035	67.25897	0.2363
35	15.174	VV	0.1244	2.76022e4	3643.65601	32.4545
36	15.475	VV	0.0882	1.83072e4	3224.55542	21.5254
37	15.801	VV	0.1053	138.47232	18.08764	0.1628
38	15.973	VV	0.1659	211.23981	17.61685	0.2484
39	16.297	VV	0.0881	170.95088	27.64967	0.2010
40	16.410	VV	0.1104	338.32788	43.68308	0.3978
41	16.666	VV	0.1004	569.44769	84.77116	0.6696
42	16.858	VV	0.1046	145.72891	20.09481	0.1713
43	17.084	VV	0.0922	1.24239e4	2006.59534	14.6079
44	17.435	VV	0.1222	289.19464	33.68008	0.3400
45	17.644	VV	0.1152	263.17422	32.24197	0.3094
46	17.842	VV	0.1029	299.21060	42.10479	0.3518
47	17.997	VV	0.0826	73.53163	12.88226	0.0865
48	18.242	VV	0.0842	6891.63135	1252.69873	8.1031
49	18.474	VV	0.0892	2970.08813	500.73773	3.4922
50	18.922	VV	0.1581	133.82294	12.58575	0.1573
51	19.100	VB	0.1147	718.22028	88.47446	0.8445
52	19.717	BV	0.1460	57.08088	5.36960	0.0671
53	20.168	VV	0.0913	2058.20264	346.41757	2.4200
54	20.305	VV	0.0921	1891.26685	305.85934	2.2237
55	20.528	VV	0.1056	2215.69312	309.28629	2.6052
56	21.135	VV	0.1904	72.62638	5.55921	0.0854
57	21.406	VB	0.1782	380.09946	30.33176	0.4469
58	22.163	BV	0.2004	47.24237	3.39352	0.0555
59	22.391	VV	0.1376	53.34341	5.38527	0.0627
60	22.595	VV	0.0829	9.22242	1.60906	0.0108
61	22.730	VP	0.1059	14.34193	2.09306	0.0169
62	23.033	VB	0.1182	386.67044	47.89508	0.4546
63	24.881	PB	0.1052	143.14851	21.08845	0.1683
64	25.624	VV	0.0907	6.02301	1.02260	7.082e-3
65	25.783	VP	0.1513	11.55310	1.07554	0.0136
66	26.800	PB	0.1546	12.24932	1.22604	0.0144
67	28.237	BB	0.2173	294.18195	18.32821	0.3459
68	32.902	BB	0.3514	220.48587	9.40924	0.2592